Synthesis and Aromatase Inhibitory Activity of Flavanones

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Purpose. Aromatase inhibitors are known to prevent the conversion of androgens to estrogens and play a significant role in the treatment of estrogen dependent diseases such as breast cancer. Some flavonoids have been reported as potent aromatase inhibitors; therefore, in an effort to develop novel anti breast cancer agents, B ring substituted flavanones with a 7-methoxy group on A ring were synthesized and tested to assess their ability to inhibit aromatase activity and to determine the optimal B ring substitution pattern.

Methods. A series of flavanones was prepared by cyclisation of 2'-hydroxychalcones previously obtained by Claisen-Schmidt condensation and the aromatase inhibitory activity of these compounds was investigated using human placental microsomes and radiolabeled [1,2,6,7-^{3}H]-androstenedione as substrate.

Results. Almost all flavanones exhibited inhibitory effect on the aromatase activity but their potency was dependent on their B ring substitution pattern. Hydroxylation at position 3′ and/or 4' enhanced the anti-aromatase activity; thus, 3′,4'-dihydroxy-7-methoxyflavanone was found to be twice more potent than aminoglutethimide, the first aromatase inhibitor clinically used.

Conclusions. These results indicated that these flavanones could be considered as potential anti breast cancer agents through the inhibition of aromatase activity and allowed us to select some of these compounds as skeleton for the development of flavonoid structurally-related aromatase inhibitors.

KEY WORDS: flavanones; aromatase; breast cancer; structure-activity relationships.

INTRODUCTION

Aromatase, which catalyses the final step in the steroidogenesis pathway of estrogens, has been the target for the design of inhibitors as agents in the treatment of breast cancer for postmenopausal women. Several classes of aromatase inhibitors such as substrate androstenedione analogs, nonsteroidal aminoglutethimide and imidazole or triazole derivatives have been designed over the past twenty years (1).

Besides the development of synthetic compounds, the potential of various classes of natural products to inhibit aromatase was evaluated in order to discover novel breast cancer chemopreventive agents. As a result, several naturally occurring and synthetic flavonoids, which are ubiquitous natural phenolic compounds and mediate a host of biologic activities (2), were found to demonstrate inhibitory effects on aromatase (3–6). The aim of the present study was to investigate the structural requirements on B-ring of flavanones for inhibition of aromatase activity, both to identify an optimal candidate among synthesized compounds, as well as to ascertain potential directions for synthetic lead-optimization studies. It has been previously demonstrated that 7-methoxyflavanone was a potent aromatase inhibitor (7) and also an effective antiproliferative agent against MCF-7 breast cancer cells (8). Moreover, this flavanone was found to be non-estrogenic unlike other flavonoids (9,10). Therefore, we undertook the pharmacomodulation of this lead. In particular, the substitution of its B-ring by hydroxy and methoxy groups was carried out and the influence of halogen atoms and dimethylamino or cyano groups on the 4'-position was also investigated. Finally, the structure-activity relationships were discussed.

Chemistry

The synthesis of flavanones **1b-23b** called on the following sequence (Scheme 1). The Claisen-Schmidt condensation of 2-hydroxy-4-methoxyacetophenone with appropriate benzaldehydes afforded various 2'-hydroxychalcones **1a-23a** (Table I). Benzaldehydes with a 4-hydroxy group and the 3,5-dihydroxybenzaldehyde were protected as tetrahydropyranyl ethers before condensation. 2'-hydroxychalcones reacted with H_2SO_4 / MeOH to give corresponding flavanones **1b-23b** (Table I) in a good yield. Structural analysis for compounds **1b-23b** are based on mass spectrometry (Table I), ^{1}H-NMR (Table II) and ^{13}C-NMR (Table III) data.

MATERIALS AND METHODS

General Experimental Procedures

NMR spectra were recorded on a Bruker 400 MHz spectrometer with Me_4Si as internal standard. ESI-MS spectra were performed on a Waters Alliance system equipped with an API-MS interface. Compounds were purified by preparative thin layer chromatography on Macherey-Nagel silica gel.

General Procedure for Obtaining Chalcones and Flavanones

2'- hydroxy-2,4'-dimethoxychalcone (1a)

2-hydroxy-4-methoxyacetophenone (0.136 g, 0.82 mmol) and 2-methoxybenzaldehyde (1.2 eq) were dissolved in EtOH and partially dehydrated barium hydroxide octahydrate (0.2 g) was slowly added. The reaction mixture was stirred at reflux for 2 h and then concentrated *in vacuo*. Water was added to the mixture that was acidified with 2M HCl (→ pH 5) and extracted with diethyl ether. The organic layer was separated, washed with water, dried over Na_2SO_4 and evaporated *in vacuo*. Purification of the residue via preparative TLC (Hexane 19 / EtOAc 1) yielded 2'-hydroxy-2,4'-dimethoxychalcone (0.155 g, 0.55 mmol).The same procedure was applied to obtain all the chalcone derivatives except for compounds **4a**, **6a**, **8a,** and **9a**.

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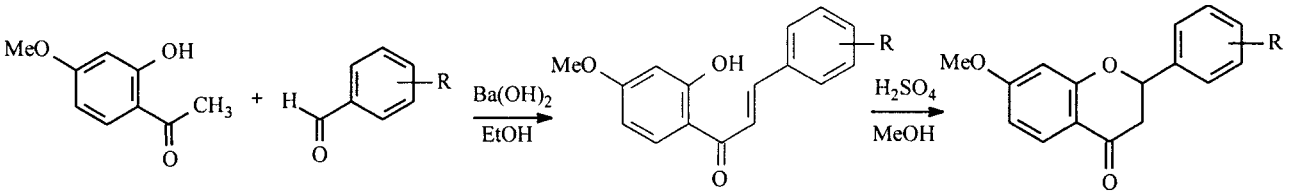


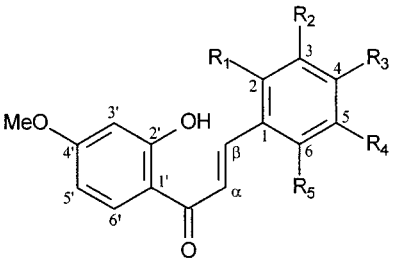
1a – 23a **1b – 23b**

Scheme 1.

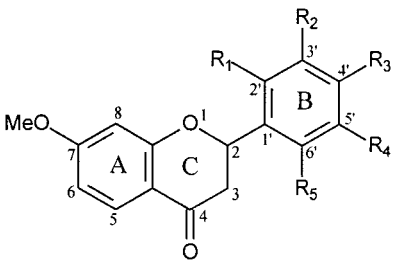
2',4 –dihydroxy-4'-methoxychalcone (4a)

4-hydroxybenzaldehyde (0.1 g, 0.82 mmol) and pyridinium *p*-toluenesulfonate (0.02 mmol) were dissolved in methylene chloride (10 ml) and 3,4-dihydro-α-pyran (0.206 g, 2.4 mmol) in methylene chloride (5 ml) was added dropwise. The reaction mixture was stirred at room temperature for 24 h, then washed with water, dried on Na_2SO_4 and evaporated *in vacuo*. The residue was purified by preparative TLC (Hexane 8 / EtOAc 2) to obtain the corresponding 4-(tetrahydropyran-2-yloxy)benzaldehyde (0.075 g, 0.36 mmol). 2-hydroxy-4-methoxyacetophenone (0.05 g, 0.3 mmol) and 4-(tetrahydropyran-2-yloxy)benzaldehyde (1.2 eq) were treated as in **1a** to give the 2'-hydroxy-4'-methoxy-4-(tetrahydropyran-2-yloxy)chalcone. This compound (0.05 g) and *p*-toluenesulfonic acid (3 mg, 0.1 eq) were dissolved in MeOH (20 ml). The reaction mixture was stirred for 4 h at room temperature and then evaporated *in vacuo*. After water had been added, the mixture was neutralized with 5% $NaHCO_3$ and extracted with EtOAc. The organic layer was separated, washed with water, dried over Na_2SO_4 and evaporated *in vacuo*. Purification of the residue via preparative TLC (Tolu-

Table I. Chalcones **1a-23a** and Flavanones **1b-23b**



1a-23a



1b-23b

Compound	R_1	R_2	R_3	R_4	R_5	Chalcones (a) Yield (%)	Flavanones (b) Yield (%)	Flavanones (b) m/z
1	OMe	H	H	H	H	67	60	284
2	H	OH	H	H	H	50	62	270
3	H	OMe	H	H	H	54	50	284
4	H	H	OH	H	H	37	67	270
5	H	H	OMe	H	H	58	62	284
6	H	OH	OH	H	H	35	30	286
7	H	OH	OMe	H	H	50	52	300
8	H	OMe	OH	H	H	24	30	300
9	H	OH	H	OH	H	28	62	286
10	OMe	OMe	H	H	H	59	50	314
11	OMe	H	OMe	H	H	48	20	314
12	OMe	H	H	OMe	H	36	50	314
13	OMe	H	H	H	OMe	20	30	314
14	H	OMe	OMe	H	H	50	43	314
15	H	OMe	H	OMe	H	30	70	314
16	OMe	OMe	OMe	H	H	33	52	344
17	OMe	H	OMe	OMe	H	50	40	344
18	H	OMe	OMe	OMe	H	70	82	344
19	H	H	Br	H	H	84	65	333
20	H	H	Cl	H	H	72	63	288
21	H	H	F	H	H	85	69	272
22	H	H	CN	H	H	86	42	279
23	H	H	$N(CH_3)_2$	H	H	66	68	297

Table II. ^{1}H NMR Data for Flavanone Derivatives

Compound	H-2′	H-3′	H-4′	H-5′	H-6′	H-2	H-3ax	H-3eq	H-5	H-6	H-8	OCH_3	$N(CH_3)_2$
1b*	/	6.92 (br d)	7.34 (br dt)	7.05 (br t)	7.62 (dd)	5.85 (dd)	2.87 (dd)	2.92 (dd)	7.88 (d)	6.61 (dd)	6.52 (d)	3.83, 3.84	
2b#	6.93 (br d)	/	6.77 (m)	7.21 (t)	6.94 (m)	5.45 (dd)	3.01 (dd)	2.78 (dd)	7.79 (d)	6.64 (dd)	6.57 (d)	3.85	
3b#	7.07 (br d)	/	6.92 (m)	7.32 (t)	7.06 (m)	5.50 (dd)	3.04 (dd)	2.79 (dd)	7.79 (d)	6.64 (dd)	6.57 (d)	3.81, 3.85	
4b*	7.35 (d)	6.90 (d)	/	6.90 (d)	7.35 (d)	5.41 (dd)	3.06 (dd)	2.81 (dd)	7.87 (d)	6.61 (dd)	6.48 (d)	3.83	
5b*	7.40 (d)	6.96 (d)	/	6.96 (d)	7.40 (d)	5.42 (dd)	3.06 (dd)	2.80 (dd)	7.87 (d)	6.61 (dd)	6.48 (d)	3.83, 3.84	
6b#	6.93 (d)	/	/	6.78 (d)	6.81 (dd)	5.36 (dd)	3.04 (dd)	2.72 (dd)	7.78 (d)	6.63 (dd)	6.53 (d)	3.84	
7b*	7.07 (d)	/	/	6.88 (d)	6.95 (dd)	5.38 (dd)	3.03 (dd)	2.79 (dd)	7.86 (d)	6.61 (dd)	6.48 (d)	3.83, 3.92	
8b*	7.00 (br s)	/	/	6.95 (d)	6.97 (dd)	5.39 (dd)	3.05 (dd)	2.80 (dd)	7.87 (d)	6.62 (dd)	6.49 (d)	3.84, 3.94	
9b*	6.42 (d)	/	6.25 (t)	/	6.42 (d)	5.36 (dd)	2.97 (dd)	2.76 (dd)	7.77 (d)	6.63 (dd)	6.56 (d)	3.85	
10b*	/	/	6.92 (dd)	7.15 (t)	7.19 (dd)	5.83 (dd)	3.00 (dd)	2.82 (dd)	7.89 (d)	6.62 (dd)	6.49 (d)	3.84, 3.88, 3.90	
11b*	/	6.50 (d)	/	6.57 (dd)	7.48 (d)	5.78 (dd)	2.95 (dd)	2.83 (dd)	7.87 (d)	6.61 (dd)	6.49 (d)	3.81, 3.83, 3.84	
12b*	/	6.85 (m)	6.85 (m)	/	7.21 (br s)	5.81 (dd)	2.84 (dd)	2.91 (dd)	7.88 (d)	6.62 (dd)	6.52 (d)	3.79, 3.82, 3.85	
13b*	/	6.61 (d)	7.30 (t)	6.61 (d)	/	6.15 (dd)	3.86 (dd)	2.54 (dd)	7.89 (d)	6.58 (dd)	6.47 (d)	3.80, 3.82, 3.82	
14b*	7.02 (br s)	/	/	6.91 (d)	7.01 (dd)	5.41 (dd)	3.07 (dd)	2.81 (dd)	7.87 (d)	6.62 (dd)	6.50 (d)	3.84, 3.91, 3.93	
15b*	6.62 (d)	/	6.47 (t)	/	6.62 (d)	5.40 (dd)	3.01 (dd)	2.82 (dd)	7.87 (d)	6.62 (dd)	6.51 (d)	3.82, 3.82, 3.84	
16b*	/	/	/	6.75 (d)	7.23 (d)	5.73 (dd)	3.04 (dd)	2.78 (dd)	7.89 (d)	6.62 (dd)	6.47 (d)	3.83, 3.89, 3.90, 3.93	
17b*	/	6.56 (s)	/	/	7.13 (s)	5.81 (dd)	2.93 (dd)	2.81 (dd)	7.88 (d)	6.61 (dd)	6.51 (d)	3.82, 3.84, 3.90, 3.92	
18b*	6.70 (s)	/	/	/	6.70 (s)	5.39 (dd)	3.04 (dd)	2.82 (dd)	7.88 (d)	6.63 (dd)	6.52 (d)	3.85, 3.87, 3.90, 3.90	
19b*	7.36 (d)	7.57 (d)	/	7.57 (d)	7.36 (d)	5.44 (dd)	2.98 (dd)	2.82 (dd0	7.87 (d)	6.63 (dd)	6.49 (d)	3.85	
20b*	7.43 (d)	7.40 (d)	/	7.40 (d)	7.43 (d)	5.46 (dd)	2.99 (dd)	2.82 (dd)	7.87 (d)	6.63 (dd)	6.50 (d)	3.85	
21b*	7.46 (d)	7.12 (d)	/	7.12 (d)	7.46 (d)	5.46 (dd)	3.01 (dd)	2.82 (dd)	7.87 (d)	6.63 (dd)	6.49 (d)	3.84	
22b*	7.61 (d)	7.74 (d)	/	7.74 (d)	7.61 (d)	5.54 (dd)	2.96 (dd)	2.86 (dd)	7.88 (d)	6.65 (dd)	6.52 (d)	3.86	
23b*	7.34 (d)	6.76 (d)	/	6.76 (d)	7.34 (d)	5.37 (dd)	3.10 (dd)	2.78 (dd)	7.86 (d)	6.59 (dd)	6.47 (d)	3.82	2.98 (s)

* measured in $CDCl_3$.
# Measured in CD_3OD.

Table III. ^{13}C NMR Data for Flavanone Derivatives

Compound	C-2	C-3	C-4	C-4a	C-5	C-6	C-7	C-8	C-8a	C-1′	C-2′	C-3′	C-4′	C-5′	C-6′	OCH_3	CN	$N(CH_3)_2$
1b*	75.2	43.6	191.6	115.2	129.0	110.3	166.2	101.1	164.2	127.8	156.1	110.8	129.6	121.1	126.6	55.6, 55.8		
2b#	81.1	45.1	193.1	115.9	129.5	111.2	168.2	102.1	165.4	142.0	114.1	158.9	116.5	130.8	118.3	56.3		
3b#	81.1	45.2	193.0	115.9	129.5	111.3	168.2	102.1	165.3	142.1	113.0	161.5	115.0	130.8	119.5	55.8, 56.3		
4b*	80.0	44.3	191.4	115.0	129.0	110.5	166.5	101.1	163.9	131.1	128.2	115.9	156.4	115.9	128.2	55.9		
5b*	80.0	44.3	191.1	115.0	129.0	110.4	166.4	101.1	160.2	131.1	128.0	114.4	163.8	114.4	128.0	55.6, 55.9		
6b#	81.3	45.0	193.6	115.8	129.5	111.1	168.2	102.0	165.6	132.0	114.8	146.6	146.9	116.3	119.3	56.3		
7b*	79.7	44.1	190.8	114.8	128.7	110.2	166.2	100.9	163.6	132.0	112.7	145.9	146.9	110.7	118.2	55.7, 56.1		
8b*	80.4	44.6	191.0	115.1	129.0	110.5	166.4	101.2	163.8	130.9	109.1	147.0	146.4	114.8	119.9	55.9, 56.3		
9b#	81.1	45.1	193.2	115.9	129.5	111.2	168.2	102.1	165.4	142.8	105.7	160.0	103.6	160.0	105.7	56.3		
10b*	75.2	43.7	191.0	114.9	128.8	110.1	166.1	100.9	163.8	132.7	145.2	152.7	112.7	124.5	118.4	55.7, 55.9, 61.1		
11b*	74.8	43.4	191.6	114.9	128.7	110.0	166.0	100.9	164.1	119.9	157.3	98.5	161.0	104.5	127.5	55.4, 55.5, 55.6		
12b*	75.0	43.4	191.2	115.0	128.8	110.1	166.1	101.0	163.9	128.6	153.9	111.6	113.7	150.0	112.6	55.7, 55.9, 55.9		
13b*	72.2	40.7	192.6	114.9	130.6	109.8	165.8	100.8	164.7	113.8	159.2	104.5	128.8	104.5	159.2	55.6, 55.9, 55.9		
14b*	80.0	44.3	190.7	114.9	128.8	109.5	166.2	101.0	163.5	131.3	111.2	149.3	149.5	110.3	118.9	55.7, 56.0, 56.0		
15b*	80.0	44.5	190.5	114.9	128.8	110.3	166.2	101.0	163.4	141.1	104.2	161.2	100.4	161.2	104.2	55.5, 55.7, 55.7		
16b*	75.1	43.6	191.2	114.9	128.8	110.0	166.1	100.9	163.4	124.9	151.3	142.2	154.2	107.5	121.4	55.6, 56.1, 60.8, 61.4		
17b*	74.8	43.6	191.4	115.0	128.8	110.1	166.0	101.0	164.0	118.7	150.5	97.3	149.8	143.4	110.5	55.6, 56.2, 56.3, 56.7		
18b*	80.3	44.5	190.5	114.8	128.8	110.3	166.2	101.0	163.4	134.4	103.3	153.6	138.3	153.6	103.3	55.7, 56.2, 56.2, 60.9		
19b*	79.2	44.2	190.1	114.8	128.8	110.4	166.3	101.0	163.3	137.9	127.8	132.0	122.7	132.0	127.8	55.7		
20b*	79.2	44.3	190.2	114.8	128.8	110.4	166.3	101.0	163.3	134.6	129.1	127.5	137.4	127.5	129.1	55.7		
21b*	79.3	44.3	190.3	114.8	128.8	110.4	166.3	100.9	163.4	134.7	128.0	115.8	163.0	115.8	128.0	55.7		
22b*	78.9	44.2	189.4	114.8	128.9	110.6	166.4	101.0	162.9	144.0	126.6	132.7	112.6	132.7	126.6	55.8	118.4	
23b*	80.2	43.9	191.4	114.9	128.7	110.0	166.1	100.9	163.9	126.0	127.6	112.4	151.0	112.4	127.6	55.6		40.5

* Measured in $CDCl_3$.
# Measured in CD_3OD.

ene 19 / Et_2O 1) afforded 2',4-dihydroxy-4'-methoxychalcone **4a** (0.03 g, 0.11 mmol).

The same procedure was applied to obtain chalcones **6a**, **8a,** and **9a** starting from respectively 3,4-dihydroxybenzaldehyde, 4-hydroxy-3-methoxybenzaldehyde and 3,5-dihydroxybenzaldehyde.

4'-hydroxy-7-methoxyflavanone (4b)

A mixture of 2',4-dihydroxy-4'-methoxychalcone (0.03g, 0.11 mmol) and 50 ml of 10% H_2SO_4 / MeOH was refluxed for 7 h and then evaporated *in vacuo*. Water was added to the mixture which was neutralized with 5% $NaHCO_3$ and extracted with EtOAc. The organic layer was separated, washed with water, dried over Na_2SO_4 and evaporated *in vacuo*. Purification of the residue via preparative TLC (Toluene 8 / Et_2O 2) afforded 4'-hydroxy-7-methoxyflavanone (0.02 g, 0.074 mmol).

The same procedure was applied to obtain all the flavanone derivatives starting from appropriate 2'-hydroxychalcones.

Biological Methods

Preparation of Human Placental Microsomes

Microsomes were obtained from human placenta after normal full-term delivery, prepared as previously described (11) and stored at –80°C.

Aromatase Assay

All enzymatic studies were performed in 0.1 M phosphate buffer (0.1 M KH_2PO_4, 1 mM dithiothreitol DTT, pH 7.4). The final incubation volume was 1 ml. Aromatase incubation mixture contained 3 mM glucose-6-phosphate, 0.5 mM NADP, 0.1 unit glucose-6-phosphate dehydrogenase, 40 nM [1,2,6,7-^{3}H] androstenedione and 0.05 mg microsomal protein. Stock solutions of each inhibitor were freshly prepared in ethanol and then diluted into the culture medium. The steroids were incubated at 37°C, extracted, identified and quantified as previously described (7).

Percentage of Inhibition Measurement

Aromatase inhibition percentage corresponded to the percentage of unchanged radiolabeled androstenedione. Values reported represented the average of two separate determinations. IC_{50} values were obtained by graphical determination.

RESULTS

In a previous work (12), we demonstrated that some chalcones were potent inhibitors of aromatase activity and that hydroxylations at position 2', 4' and 6' on A ring may be required to obtain high aromatase inhibitory effect. In contrast, we showed that the presence of a 4'-methoxy group decreased the anti-aromatase activity. Therefore, the chalcones here synthesized (**1a**-**23a**), which had this type of substitution, were not further evaluated.

The anti-aromatase activities of flavanones **1b**-**23b** are reported in Table IV. Aminoglutethimide (**AG**, IC_{50} = 5.2 μM) and 7-methoxyflavanone (**7-OMe F**, IC_{50} = 8.0 μM) were used as reference compounds.

Table IV. Inhibition of Human Placental Aromatase by Flavanones

Compound	IC_{50} (μM)[a]
AG	5.2
7-OMe F	8.0
1b	6.6
2b	3.5
3b	(43.6)
4b	3.7
4b	(43.8)
6b	2.5
7b	6.2
8b	5.4
9b	3.5
10b	6.6
11b	8.0
12b	(26.3)
13b	(25.7)
14b	(42.7)
15b	(31.9)
16b	4.8
17b	(29.3)
18b	(33.9)
19b	(41.2)
20b	(47.3)
21b	(42.3)
22b	(38.3)
23b	(16.6)

[a] When 50% inhibition could not be reached at 10 μM, the % of inhibition is given in parentheses.

All the flavanones tested caused dose-dependent inhibition of aromatase activity but their potency was dependent on the B ring substitution pattern. Compounds **2b**, **4b**, **6b,** and **9b**, the most active flavanones, were found to be more potent than aminoglutethimide and 7-methoxyflavanone. Therefore, the B ring substitution by hydroxy groups enhanced the inhibitory effect on aromatase activity. The 3′ and 4'-hydroxy groups seemed to act synergistically since flavanone **6b** is more potent than flavanones **2b** and **4b**. On the contrary, methylation of these hydroxy groups decreased the inhibitory effect since flavanones **3b**, **5b**, **14b,** and **15b** appeared to be weak aromatase inhibitors relative to their hydroxylated counterparts. Surprisingly, the presence of a 2'-methoxy group seemed to be responsible for an increase in anti-aromatase activity; the importance of such a substituent was suggested by comparison of activities of flavanones **1b**, **10b**, **11b,** and **16b** respectively with those of flavanones **7OMe F**, **3b**, **5b,** and **14b**, where the only difference between these two series of compounds was the presence of an additional 2'-methoxy group in the first subset. However, the weak anti-aromatase activity of flavanones **12b** and **13b** relative to the 2',7-dimethoxyflavanone **1b** clearly indicated that the presence of an additional methoxy group at position 5′ or 6' drastically reduced the inhibitory effect.

Finally, contrary to a 4'-hydroxy group, the presence of halogen atoms as well as a cyano group at position 4' did not influence the inhibitory effect while the substitution by a dimethylamino group led to a less more potent flavanone.

DISCUSSION

Different studies have demonstrated that several naturally occurring flavonoids such as 7-hydroxyflavanone, 7-hydroxyflavone, naringenin (5,7,4'-trihydroxyflavanone), apigenin (5,7,4'-trihydroxyflavone), luteolin (5,7,3′,4'-tetrahydroxyflavone) or eriodictyol (5,7,3′,4'-tetrahydroxyflavanone) were potent aromatase inhibitors (3-7,12). However, most of these compounds were also found to display estrogenic activity (9,13). Structure-activity relationships clearly indicated that a 7-hydroxy group was the substituent essential for enhanced aromatase inhibitory activity but flavonoids with such a substituent were found to be invariably estrogenic.

In contrast, 7-methoxyflavanone did not display estrogenic activity (9,10) and exhibited an aromatase inhibitory effect (7) as well as an antiproliferative activity on MCF-7 breast cancer cells (8). These data may suggest that pharmacomodulation of the B ring of this flavanone could lead to compounds having a balance between different anti breast cancer activities.

The present results demonstrated that some of the synthesized flavanones such as compounds **2b**, **4b**, **6b,** or **9b** were potent aromatase inhibitors which indicated that hydroxylations on B ring may be required to obtain high aromatase inhibitory effect. Thus, the activity exhibited by 3′-hydroxy-7-methoxyflavanone **2b** and 3′,4'-dihydroxy-7-methoxyflavanone **6b** relative to 7-methoxyflavanone and 4'-hydroxy-7-methoxyflavanone **4b** respectively, clearly showed that the presence of an additional 3′-hydroxy group enhanced the anti-aromatase activity. These findings are consistent with the increased potency we observed with eriodictyol (5,7,3′,4'-tetrahydroxyflavanone) relative to naringenin (5,7,4'-trihydroxyflavanone) (12). Among all the flavanones tested, 3′,4'-dihydroxy-7-methoxyflavanone **6b** was found to be the most active and twice more potent than aminoglutethimide, the first aromatase inhibitor clinically used. Therefore, structure-activity relationships with flavanones seem to indicate that two hydroxy groups at position 3′ and 4' are the optimal pattern of B ring substitution that gives rise to anti-aromatase activity.

We also demonstrated that the presence of halogen atoms or a cyano group on the 4'-position of B ring of flavanones did not influence aromatase inhibitory effect whereas the results for the aromatase inhibition by azole-type inhibitors showed the importance of such a *para*-substituted phenyl moiety in the interaction with the active site of aromatase (14).

Further experiments are currently undergoing to examine both the ability of the most active flavanones to inhibit the MCF-7 breast cancer cells growth and to interact with estrogen receptor since hydroxy substituents on flavonoid nucleus were found to influence estrogenic activity (9,13). Thus, Miksicek demonstrated that flavonoids with a 4'-hydroxy group were invariably estrogenic whereas hydroxylations that create catechols abolished estrogenic effect (13). Therefore, besides the potent aromatase inhibitory effect exhibited by 3′,4'-dihydroxy-7-methoxyflavanone, this compound might be without estrogenic activity and could actually be considered as a potential anti-breast cancer agent.

Finally, on the basis of the studies reported herein, 3′-hydroxy-7-methoxyflavanone **2b** and 3′,4'-dihydroxy-7-methoxyflavanone **6b** have been selected for further modulation on C ring in order to develop flavonoid structurally-related aromatase inhibitors.

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