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New heterocyclic derivatives of benzimidazole with germicidal activity
Part XIII. In vitro aromatase inhibitory activity; preliminary observations

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Abstract

Several benzimidazole derivatives have been tested in vitro for their ability to inhibit human placental aromatase activity. The results show that five out of the six compounds tested are more active in the inhibition of aromatase activity than aminoglutethimide (AG), which we chose as the reference compound and which, as an aromatase inhibitor, is currently being used in the treatment of metastatic breast cancer.

Keywords: Aromatase; Inhibitory activity; Benzimidazoles

1. Introduction

From 1980 our research group synthesized and tested the biological activity of a large number of benzimidazole and benzoxazole derivatives [1–13]. The most interesting compound was 5-fluoro-2-(5′-nitro-2′-furyl) benzimidazole ($FONO_2$) which displayed very good in vitro and in vivo antibacterial and antimycotic activity [14].

At the same time several series of steroidal and non-steroidal compounds have been tested for their inhibitory activity on aromatase enzyme, since aromatase inhibitors are very useful in the therapy of some estrogen-dependent cancers (i.e. breast cancer) [15].

A study, developed on a wide variety of heterocyclic compounds, showed the correlation between antimycotic activity and aromatase inhibitory activity of some compounds [16]. Moreover, several series of imidazole and benzimidazole derivatives, recently synthesized [17], have shown an aromatase inhitibory activity comparable or larger than that of aminoglutethimide (AG) which is widely studied [18a–c] and currently used in the treatment of breast cancer [15] for its ability to inhibit estrogen biosynthesis.

On these bases we have been testing six benzimidazole derivatives as inhibitors of human placenta aromatase activity.

Two of the compounds tested have been newly synthesized for this work with a structure similar to some heterocyclic compounds which have shown good aromatase inhibitory activity (Scheme 1).

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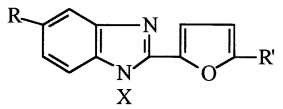


(1) R = H, R' = H, X = H (2) R = H, R' = NO_2, X = H

(3) R = F, R' = NO_2, X = H (4) R = F, R' = NO_2, X = CH_3CH_2

(5) R = H, R' = NO_2, X = C_6H_5CO (6) R = F, R' = NO_2, X = C_6H_5CO

Scheme 1.

2. Experimental

2.1. Chemistry

Melting points were determined on a Büchi 520 apparatus and are uncorrected. ^{1}H NMR spectra were recorded with a Bruker AW-80 and the chemical shift values (ppm) are relative to tetramethylsilane as internal standard. The IR spectrophotometer was a Perkin Elmer 710 B. The mass spectrometer was a Varian MAT 311 A. Precoated Kieselgel 60 F_{254} plates (Merck) were used for TLC.

Compounds 2-(2′-furyl) benzimidazole (**1**) [1], 2-(5′-nitro-2′-furyl) benzimidazole (**2**) [8], 5-fluoro-2-(5′-nitro-2′-furyl) benzimidazole (**3**) [1] and *N*-ethyl-5-fluoro-2-(5′-nitro-2′-furyl) benzimidazole (**4**) [3] have already been synthesized in our laboratory, while the new derivatives *N*-benzoyl-2-(5′-nitro-2′-furyl) benzimidazole (**5**) and *N*-benzoyl-5-fluoro-2-(5′-nitro-2′-furyl) benzimidazole (**6**) were synthesized as follows:

One mmol of the specific 1H-benzimidazole, 2 mmol of benzoyl chloride and 4 drops of triethylamine were dissolved in 20 ml of anhydrous benzene and warmed under reflux for 24 h, then the solution was washed three times with water, dried over $CaCl_2$ and evaporated under vacuum thus obtaining a solid residue which was purified by means of the fractional crystallization by a ligroin.

Table 1 shows the main physical characteristics of the new synthesized compounds; ^{1}H NMR, IR and MS data are shown in Table 2.

2.2. Microbiology

The antimicrobial activity of the benzimidazole derivatives has been assayed against three (Gram −) and five (Gram +) strains and against the mycete *Candida albicans* measuring the minimal inhibitory concentration (MIC) with the dilution broth method [19] using 96 multiple well plates (Cornig Glass Works) instead of test-tubes. Table 3 reports the experimental results of this investigation.

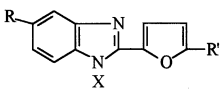
2.3. Aromatase inhibitory activity

2.3.1. Preparation of microsomes

To test the aromatase activity we used the microsomal fraction of human term placentas extracted according to Ryan's method [20], modified by Thompson and Sitieri [21]. The microsomes were finally suspended in a minimal volume of phosphate buffer (0.05 mol, pH 7.5) and stored in plastic tubes (Falcon) at − 80°C. No loss of aromatase activity occurred over the study period.

Protein concentration was determined by the Bio-Rad Protein Assay Kit.

Table 1
Characteristics of synthesized compounds



N	R	R′	X	M.p. (°C)	Color	Purification and crystallization	TLC eluant
5	H	NO_2	C_6H_5CO–	129–131	yellow	ligroin	$CHCl_3$/MeOH, 98:2
6	F	NO_2	C_6H_5CO–	127–129	yellow	ligroin	$CHCl_3$/MeOH, 98:2

Table 2
MS, IR and NMR characteristics of synthesized compounds

N	Molecular formula	Mass spectra		I.R. spectra (>CO absorption band cm^{-1})	^{1}H NMR spectra (δ) ppm[a]
		M^+	(I%)		
5	$C_{18}H_{11}N_3O_4$	333	33	1700	7.2–8.0 arom. (11H)
6	$C_{18}H_{10}N_3O_4F$	351	37	1700	6.9–7.8 arom. (10H)

[a] The benzimidazole derivatives are dissolved in $CDCl_3$.

Table 3
In vitro antibacterial and antimycotic[a] activity of benzimidazole derivatives (minimal inhibitory concentration in γ/ml)[b]

	A	B	C	D	E	F	G	H	I
1	0	0	0	0	0	0	0	0	0
2	15	15	15	3	15	7	7	15	15
3	15	15	7	3	15	7	15	15	31
4	15	31	15	3	62	500	125	15	31
5	15	31	31	3	15	31	7	15	15
6	3	62	62	20	62	125	31	15	250

[a] International strains: A, *St. epidermidis* ATCC 14990; B, *St. aureus* ATCC 9144; C, *S. lutea* ATCC 9341; D, *B. subtilis* ATCC 6051, E, *Str. faecalis* ATCC 8043; F, *S. typhimurim* ATCC 13311; G, *E. aerogenes* ATCC 13047; H, *E. coli* ATCC 10538

[b] Collection of Department of Experimental Medicine and Biochemistry Sciences of Perugia University: I, *Candida albicans*.

2.3.2. Aromatase activity assay

[^{3}H]-water released in the incubation medium by the conversion of 1-^{3}H-labeled androstenedione into estrogen was determined essentially according to the procedure of Thompson and Sitieri [21].

To each incubation tube containing 1.9 ml of phosphate buffer (67 mmol, pH 7.4), microsomal protein (25 μg) and 100 μl of NADPH (600 μmol, 0.5 g/l) (Sigma), 10 μl of inhibitors at appropriate concentrations were added. After 30 min of incubation at 37°C in a shaking water bath 10 μl of [^{3}H]-androstenedione (3.63 TBq/mmol, 37 MBq/ml) (Amersham) were added to the mixture and the incubation continued for an additional 30 min. Chloroform (3 ml) was then added to each tube and the mixture was vortexed vigorously for 40 s and centrifuged for 10 min. Suspensions of trichloroacetic acid (500 μl, 24% w/w) (Fluka) and Norit A (500 μl, 5% w/w) (Sigma) diluted in bidistilled water, were added to 1.5 ml aliquots of the aqueous layer and the samples were incubated at 37°C with shaking for 30 min. The mixture was centrifuged at 800 g for 5 min and filtered (0.45 μm, Costar) to remove Norit A. Aliquots of the filtrate (600 μl) were mixed with 6 ml of scintillation fluid (Insta-Gel Packard) and the tritium radioactivity was counted by a Tri-Carb 4000 liquid scintillation counter (Packard, IL, USA).

The blank value obtained from the incubation of all reagents except NADPH was subtracted from those with NADPH.

The results are reported in Table 4.

3. Discussion

The method herein described for measuring aromatase activity shows that among the six compounds tested, five had good inhibitory activity on the human placental aromatase. Furthermore, the *N*-benzoyl substituted derivatives **6** and **5** were respectively, 25 and 60 times more active than AG, used as the reference compound.

Table 4
In vitro inhibition of aromatase activity of benzimidazole derivatives and aminoglutethimide measured by the uptake of [^{3}H]-androstenedione

N	Conc. (μM)	% Uptake	EC_{50} (μM)
1	5	85	>5
	0.5	87	
2	5	8	0.48
	0.5	45	
	0.11	80	
3	5	8	0.95
	0.5	70	
	0.11	100	
4	5	0	0.33
	0.5	33	
	0.11	77	
5	0.5	0	0.04
	0.11	35	
	0.04	54	
	0.012	68	
6	0.5	0	0.10
	0.11	59	
	0.04	71	
	0.012	111	
AG	5	0	2.50
	0.5	114	

According to Taylor et al. [16] we found that the five compounds which inhibit the aromatase activity also had good antimycotic activity, but the modulation due to the presence of substituents goes in the opposite direction. In fact, even from little experimental data, we can see that the presence of substituents at 1 and/or 5 position of the benzimidazolic ring strongly increased the inhibition of aromatase activity but did not work in the same way as antimycotic activity [3].

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