Synthesis and Antifungal Activity Against Strains of *Candida albicans* of 6-Fluoro-4(5 or 7)-Chloro-2-(Difluorobenzoyl)aminobenzothiazoles

Domenico Armenise, Nicolino De Laurentis, Antonia Reho, Antonio Rosato and Flaviano Morlacchi*

Dipartimento Farmaco-chimico, Facoltà di Farmacia, Università degli Studi di Bari, Via E. Orabona 4, 70125 Bari (Italy)

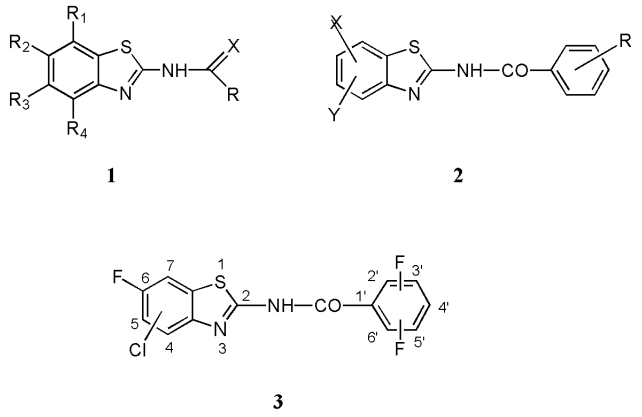
Received March 2, 2004

A series of 6-fluoro-4-(5 or 7)-chloro-2-(difluorobenzoyl)aminobenzothiazoles **3a-r** were prepared to investigate their potential biological activity. In this work, the results of their *in vitro* antifungal activity against some strains of *Candida albicans* are reported. It was found that some derivatives displayed antifungal activity higher than that for **3k** [1a] compound already described in literature.

J. Heterocyclic Chem., **41**, 771 (2004).

Introduction.

The benzothiazole heterocyclic nucleus occurs in a number of compounds endowed with interesting biological activities [1a], including antibacterial and antifungal [1b], antiparasitic, antiviral and antitubercular. Moreover, some *N*-benzothiazol-2-yl-amides or thioamides **1** (X = O, S) (Figure 1) are useful for the treatment of diseases related to the adenosine receptor [2] and treatment or prevention of diseases and/or disorders associated with the nuclear hormone receptor families [3]. In particular, some dihalogenated benzothiazoles **2** (Figure 1) substituted at the 2-position with a benzoylamino moiety showed antibacterial, antifungal and antitubercular activities [1a]. Among these last compounds, 5-chloro-6-fluoro-2',6'-difluoro-2-benzoylaminobenzothiazole **3k** was found to possess antifungal activity against *Candida albicans, Trichophyton rubrum* and *Trichophyton mentagrophytes* [1a]. Taking into account this finding and as part of our in progress research program aimed at discovering new aza-sulfurated antimicrobial agents [1b], we decided to synthesize all the regioisomers of monochloro-trifluoro-substituted 2-benzoylaminobenzothiazole compounds **3** (Figure 1), as a SAR expansion in an attempt to enhance the antifungal activity of the derivative already known **3k**, as well as to investigate their potential biological activity.



1 **2**

3

Figure 1

Results and Discussion.

Chemistry.

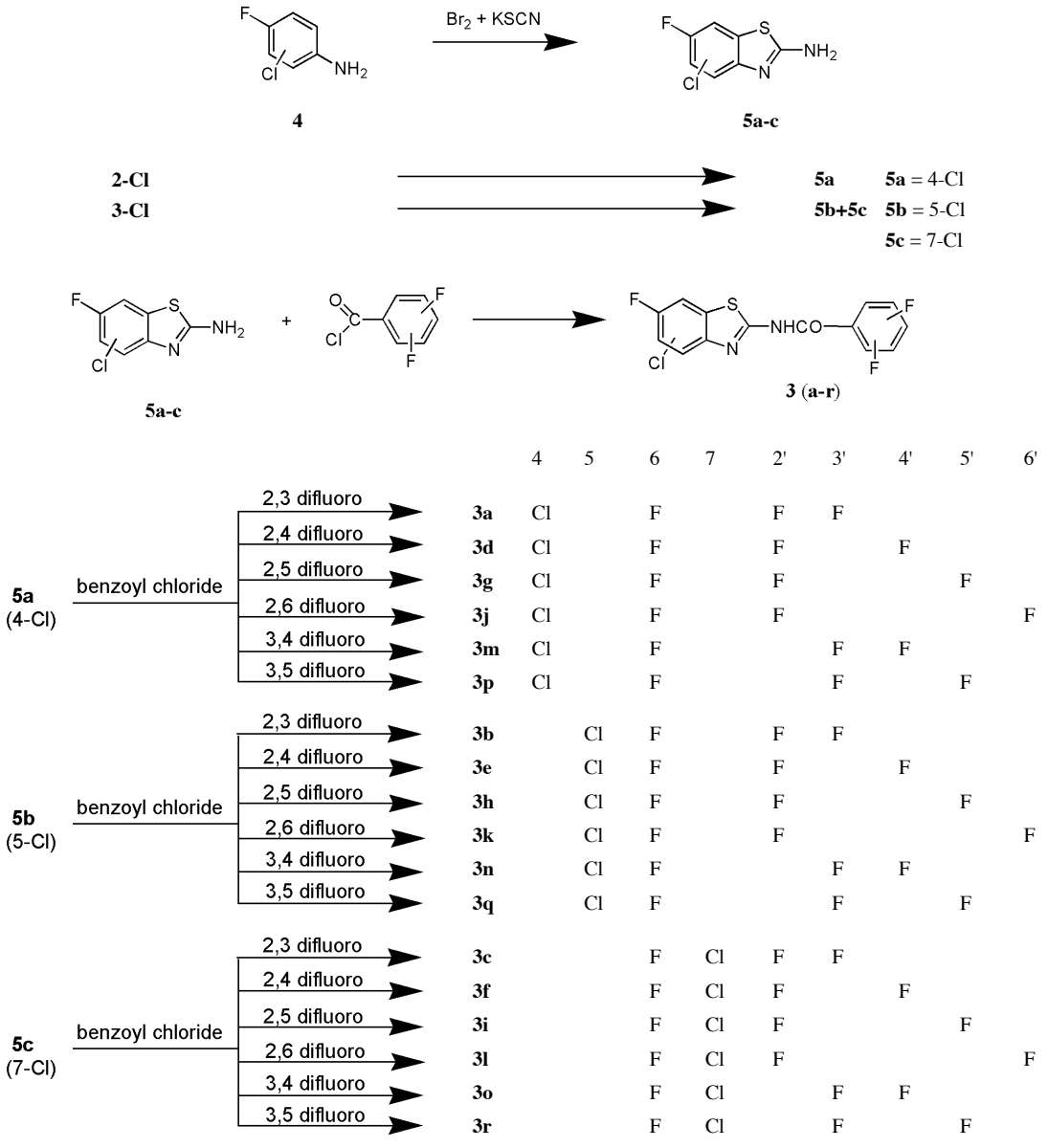
As shown in Scheme 1, the desired compounds **3a-r** were obtained following a two step procedure involving at first the synthesis of the appropriate 2-aminobenzothiazoles **5a-c** by reaction of 4-fluoro-, 2- or 3–chloroaniline **4** with bromine and potassium thiocyanate [4,5].

Next, all the possible 18 isomeric compounds **3a-r** were achieved by reaction of compounds **5a-c** with the suitable difluorobenzoyl chloride in triethylamine and dry 1,4 dioxane. In this Scheme are also specified the structures of the starting 2-aminobenzothiazoles **5a-c**, the fluoro substituted benzoyl chlorides employed and the structures of the corresponding 2-benzoylaminobenzothiazole compounds **3a-r** obtained.

The structures of the new compounds were fully supported by microanalytical and spectrographic (ir, ^{1}H nmr, and mass) data. Compound **3k** showed spectral data consistent with those reported in literature [1a]. Tables 1 - 4 show the main physical properties and spectral data for compounds **5a-c** and **3a-r**.

As for the yields obtained it should be pointed out that the 5-chloro–substituted compounds **3** were obtained in yield higher than those of 4-substituted analogues. The highest yields were observed for compounds **3n** and **3b** (*i.e.*, 87.4% and 68.1%, respectively). In some cases (*i.e.*, **3c, 3f, 3o, 3r)** the low yields obtained are due to the formation of co-products that have been removed by column chromatography and were considered outside the scope of the present work. In particular, from a synthetic point of view, the results indicate that low yields were obtained for compounds where a chloro atom at C-7 position of the benzothiazole nucleus occurs.

Scheme 1



		4	5	6	7	2'	3'	4'	5'	6'
5a (4-Cl)	**3a**	Cl		F		F	F			
	3d	Cl		F		F		F		
	3g	Cl		F		F			F	
	3j	Cl		F		F				F
	3m	Cl		F			F	F		
	3p	Cl		F			F		F	
5b (5-Cl)	**3b**		Cl	F		F	F			
	3e		Cl	F		F		F		
	3h		Cl	F		F			F	
	3k		Cl	F		F				F
	3n		Cl	F			F	F		
	3q		Cl	F			F		F	
5c (7-Cl)	**3c**			F	Cl	F	F			
	3f			F	Cl	F		F		
	3i			F	Cl	F			F	
	3l			F	Cl	F				F
	3o			F	Cl		F	F		
	3r			F	Cl		F		F	

Table 1

Yield and Physical Properties of Compounds **5**

Compd	Yield (%)	m.p. (°C)	Reference
5a	75.2	205-210	[4]
5b	30.0	229-231	
		217-220	[5]
5c	21.7	198-201	
		189-192	[5]

Microbiology.

The *in vitro* antifungal assays of the prepared dihalogenated benzothiazole **3a-r** compounds were carried out (see Table 5a) against some strains of the fungus *Candida albicans*. Furthermore, taking into account that sorbic acid is widely used in food manufactoring due to its fungistatic activity, we chosen that compound as reference. The fungal strains were grown in Sabouraud dextrose agar. In this context, it should be remembered that an interesting antimicrobial activity against *Candida albicans* has previously been claimed for compound **3k** [1a], although the exact strains used were not specified.

Table 5a shows the results obtained in our microbiologi-

Table 2

Yield, Elemental Analyses and Physical Properties of Compounds **3a-r**

Compd	Yield (%)	(*)	Anal. Calcd. % for $C_{14}H_6ClF_3N_2OS$ C 49.06	H 1.76	N 8.17	m.p. (°C)	i.r.(nujol) ν(cm^{-1})	R_f
			Found					
3a	58.0	W	49.19	2.12	8.00	225-228	3412, 1676	0.47
3b	68.1	W	48.94	2.10	7.78	260-262	3403, 1677	0.52
3c	5.9	W	49.43	2.14	7.82	261-264	3403, 1678	0.53
3d	42.6	Y	49.25	2.16	8.40	274-277	3415, 1670	0.78
3e	14.6	W	48.70	2.15	8.50	264-267	3401, 1673	0.82
3f	3.0	W	48.71	2.12	8.53	235-242	3423. 1678	0.61
3g	66.2	Y	48.68	2.07	8.01	261-265	3414, 1677	0.73
3h	36.2	W	48.69	1.87	7.80	362-365	3401, 1669	0.79
3i	22.2	W	49.28	2.11	7.90	188-190	3412, 1679	0.54
3j	21.7	W	48.93	2.14	7.86	243-247	3412, 1697	0.29
3k	63.0	W	48.69	2.06	8.20	267-271	3400, 1684	0.32
3l	29.1	W	49.43	2.09	7.77	260-266	3200, 1693	0.43
3m	65.2	Y	48.67	2.10	8.00	222-225	3239, 1675	0.63
3n	87.4	W	49.29	1.94	7.90	245-250	3448, 1680	0.72
3o	9.5	W	48.80	2.00	8.00	275-278	3200, 1671	0.54
3p	19.1	Y	48.70	2.10	8.13	260-262	3416, 1682	0.78
3q	36.2	W	49.10	1.98	8.02	228-230	3190, 1695	0.67
3r	6.0	W	48.76	2.13	7.79	198-208	3190. 1695	0.84

(*) W = white solide; Y = yellow solide; R_f: Tlc (hexane-ethyl acetate 7:3 v/v).

Table 3

^{1}H nmr Data of Compounds **5** [a]

5a (DMSO-d_6) δ 7.25 (d, 1 H, J = 9.0 Hz, H_7-aromatic), 7.59 (d, 1 H, J = 7.0 Hz, H_5-aromatic), 7.8 (s, broad, 2 H, NH_2).

5b ($CDCl_3$) δ 5.18 (s, broad, 2 H, NH_2), 7.29 (d, 1 H, J = 8.0 Hz, H_4-aromatic), 7.46 (d, 1 H, J = 6.0 Hz, H_7-aromatic). (DMSO-d_6) δ 7.55 (d, 1 H, $J_{H\text{-}F}$ = 6.0 Hz, H_4), 8.00 (d, 1 H, $J_{H\text{-}F}$ = 9.0 Hz, H_7), 9.75 (s, br, 2 H, NH_2) [5].

5c ($CDCl_3$) δ 5.3 (s, broad, 2 H, NH_2), 7.12 (t, 1 H, J = 10.0 Hz, H_4-aromatic), 7.34 and 7.38 (dd, 1 H, J = 5.0 Hz, H_5-aromatic). (DMSO-d_6) δ 6.93-7.28 (m, 2 H, aromatics), 7.60 (s, br, 2 H, NH_2) [5]

cal assays against four strains of *Candida albicans*. By comparing these results with those described for **3k** [1a], it is possible to conclude that 9 out of 17 compounds exhibit antifungal activities higher than that of **3k**, taking into account that in our hands **3k** showed a MIC > 500 μg/ml.

In Table 5b, an overall valuation of the antifungal activity of the regioisomers **3a-r** as a function of the halogen substitutions is specified. It is apparent from the results in this Table that the best antifungal activity occurs when the 3',5' positions of the benzoyl moiety are substituted by difluoro atoms as well as the 4 or 5 position of the ben-

Table 4

^{1}H nmr ($CDCl_3$, δ) Data of Compounds **3** [a]

3a 1.6 (s, br, 1H, NH), 7.25-7.38 (m, 2 H, $H_{5'} + H_{6'}$), 7.41-7.52 (m, 2 H, $H_7 + H_{4'}$); 7.95 (t, 1 H, H_5, J = 7.0 Hz)

3b 1.6 (s, br, 1H, NH), 7.30-7.40 (m, 1 H, $H_{5'}$), 7.48 (q, 1 H, $H_{6'}$, J = 8.0 Hz); 7.62 (d, 1 H, H_4, J = 8.0 Hz); 7.84 (d, 1 H, H_7, J = 6.0 Hz); 7.97 (t, 1 H, $H_{4'}$, J = 6.0 Hz)

3c 7.2-7.4 (m, 2 H, $H_{5'} + H_{6'}$); 7.4-7.5 (m, 1 H, H_4); 7.6-7.7 (m, 1 H, H_5); 7.9-8.1 (m, 1 H, $H_{4'}$); 10.0 (s, br, 1H, NH)

3d 7.02 (t, 1 H, $H_{6'}$, J = 10.0 Hz); 7.11 (t, 1 H, $H_{5'}$, J = 10.0 Hz); 7.28 (d, 1 H, H_7, J = 8.0 Hz); 7.47 (d, 1 H, H_5, J = 8.0 Hz); 8.28 (q, 1 H, $H_{3'}$, J = 6.0 Hz); 10.0 (s, br, 1H, NH)

3e 1.6 (s, br, 1H, NH), 6.98-7.07 (m, 1 H, $H_{6'}$), 7.12 (t, 1 H, $H_{5'}$, J=9.0 Hz); 7.61 (d, 1 H, H_4, J = 10.0 Hz); 7.84 (d, 1 H, H_7, J = 8.0 Hz); 8.28 (q, 1 H, $H_{3'}$, J = 9.0 Hz);

3f 1.8 (s, br, 1H, NH), 6.95-7.06 (m, 1 H, $H_{6'}$), 7.08-7.18 (m, 1 H, $H_{5'}$); 7.25-7.36 (m, 1 H, H_4); 7.60-7.70 (m, 1 H, H_5); 8.30 (q, 1 H, $H_{3'}$, J = 7.0 Hz);

3g 7.20-7.40 (m, 3 H, $H_7 + H_{3'} + H_{4'}$); 7.48 (d, 1 H, H_5, J = 8.0 Hz); 7.87-7.97 (m, 1 H, $H_{6'}$); 10.0 (s, br, 1H, NH)

3h 7.2-7.4 (m, 2 H, $H_{3'} + H_{4'}$); 7.63 (d, 1 H, H_4, J = 8.0 Hz); 7.85 (d, 1 H, H_7, J = 6.0 Hz); 7.9-8.0 (m, 1 H, $H_{6'}$); 10.0 (s, br, 1H, NH)

3i 7.20-7.40 (m, 3 H, $H_4 + H_{3'} + H_{4'}$); 7.6-7.7 (m, 1 H, H_5); 7.88-7.98 (m, 1 H, $H_{6'}$); 10.0 (s, br, 1H, NH)

3j 1.6 (s, br, 1H, NH); 6.92 (t, 2 H, $H_{3'} + H_{5'}$, J=8.0 Hz); 7.19 (d, 1 H, H_7, J = 8.0 Hz); 7.42 (t, 1 H, $H_{4'}$, J = 8.0 Hz); 7.47 (d, 1 H, H_5, J = 8.0 Hz)

3k 1.7 (s, br, 1H, NH); 7.02 (t, 1 H, $H_{4'}$, J=7.5 Hz); 7.43 (d, 1 H, H_4, J = 7.5 Hz); 7.47-7.55 (m, 2 H, $H_{5'} + H_{3'}$); 7.62 (d, 1 H, H_7, J = 7.5 Hz) 7.00-7.70 (m, 5H, H-aromatics), 12.8 (s, 1H, NH) [1a].

3l 1.7 (s, br, 1H, NH), 7.05 (t, 1 H, H_4, J = 8.0 Hz), 7.2-7.3 (m, 3 H, $H_5 + H_{3'} + H_{5'}$), 7.4-7.6 (m, 1 H, $H_{4'}$)

3m 7.28 (d, 1 H, H_7, J=8.0 Hz); 7.30-7.40 (m, 1 H, $H_{6'}$); 7.48 (d, 1 H, H_5 , J=7.0 Hz); 7.68-7.78 (m, 1 H, $H_{5'}$); 7.88 (t, 1H, $H_{2'}$, J= 8.0 Hz); 10.0 (s, br, 1H, NH)

3n 1.8 (s, br, 1H, NH); 7.35 (q, 1 H, $H_{6'}$, J=8.0 Hz); 7.64 (d, 1 H, H_4 , J= 8.0 Hz); 7.76 (d, 1 H, H_7 , J=6.0 Hz); 7.8-7.9 (m, 1 H, $H_{5'}$); 7.93 (t, 1H, $H_{2'}$, J= 8.0 Hz)

3o 1.8 (s, br, 1H, NH); 7.3-7.4 (m, 2 H, $H_{6'} + H_4$); 7.6-7.7 (m, 1 H, H_5); 7.8-7.9 (m, 1 H, $H_{5'}$); 7.9-8.1 (m, 1 H, $H_{2'}$)

3p 1.6 (s, br, 1H, NH); 7.10 (t, 1 H, $H_{4'}$, J=7.5 Hz); 7.30 (d, 1 H, H_7 , J= 9.0 Hz); 7.48 (d, 1 H, H_5 , J=9.0 Hz); 7.5-7.6 (m, 2 H, $H_{2'} + H_{6'}$)

3q 1.7 (s, br, 1H, NH); 7.08-7.15 (m, 1 H, $H_{4'}$); 7.60 (d, 2 H, $H_{2'} + H_{6'}$, J= 4.0 Hz); 7.62 (d, 1 H, H_4 , J=8.0 Hz); 7.78 (d, 1H, H_7 , J= 6.0 Hz)

3r 1.7 (s, br, 1H, NH); 7.05-7.15 (m, 1 H, $H_{4'}$); 7.20-7.40 (m, 1 H, H_4); 7.50-7.70 (m, 3 H, $H_5 + H_{6'} + H_{2'}$)

[a] All NH signals disappear with D_2O.

zothiazole nucleus is substituted by a chloro atom (**3p, 3q**). A lower antimicrobial activity (comparable however to that of the reference, sorbic acid) was observed when the 2',5' positions of the benzoyl moiety are substituted by difluoro atoms and the 5 position of the benzothiazole nucleus is substituted by a chloro atom (**3h**).

In conclusion, as can be seen from Tables 5a and 5b, compounds **3** show antifungal activity comparable (**3h**) or in some cases better (**3p, 3q**) than that of the reference compound sorbic acid.

Further studies are currently in progress to elucidate the biological activities of compounds **3**.

Table 5a

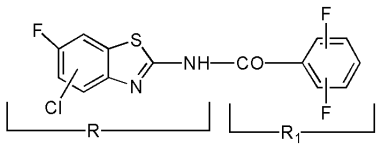
Antifungal Activities of Compounds **3a-r** against *Candida albicans* (MIC, μg/ml)

Compounds	*C. albicans* ATCC 10231	*C. albicans* ATCC 14053	*C. albicans* NRRL Y12983	*C. albicans* NRRL Y896
3a	500	500	500	500
3b	-----	-----	-----	-----
3c	500	500	250	500
3d	-----	-----	-----	-----
3e	500	500	500	500
3f	-----	-----	-----	-----
3g	500	500	500	500
3h	250	500	250	500
3i	-----	500	-----	500
3j	-----	-----	-----	-----
3k	-----	-----	-----	-----
3l	-----	-----	-----	-----
3m	500	500	500	500
3n	-----	-----	-----	-----
3o	-----	-----	-----	-----
3p	250	250	250	500
3q	250	250	250	500
3r	-----	-----	-----	-----
Sorbic Acid	250	500	250	500

--- = MIC > 500 μg/ml**.**

Table 5b

Overall valuation of the antifungal activity of regioisomers **3a-r** as a function of the substituent [halogen(s)] position [a]



3 (a-r)

R_1	R: 4-Cl		R: 5-Cl		R: 7-Cl	
	Compound	Activity	Compound	Activity	Compound	Activity
2',3'-F	**3a**	*	**3b**		**3c**	*
2',4'-F	**3d**		**3e**	*	**3f**	
2',5'-F	**3g**	*	**3h**	**	**3i**	*
2',6'-F	**3j**		**3k**		**3l**	
3',4'-F	**3m**	*	**3n**		**3o**	
3',5'-F	**3p**	***	**3q**	***	**3r**	
	Sorbic Acid	**		**		**

[a] For sake of clarity about the structure-antifungal activity relationship, in this Table the regioisomers **3a-r** are listed as a function of the substituent [halogen(s)] position. **R** indicates the position of chloro-atom on the benzothiazole nucleus, $\mathbf{R_1}$. indicates the position of the fluoro-atoms on the benzoyl nucleus. The MIC values reported in Table 5a for each of the 4 strains of *Candida albicans* , in this Table are indicated as follows: * for MIC values < sorbic acid standard, ** for MIC values = standard, *** for MIC values > standard.

EXPERIMENTAL

Chemistry.

Melting points were determined in open capillary tubes with a Büchi apparatus and are uncorrected. Ir spectra were obtained on a Perkin-Elmer 283 spectrophotometer (nujol mull or potassium bromide pellets, in each case specified). ^{1}H nmr spectra were recorded on Varian-Mercury instrument operating at 300 MHz. Chemical shifts are given in δ values (ppm) and the coupling constants are express in J values (Hz) downfield from tetramethylsilane as internal standard. Mass spectra were recorded on a Hewlett-Packard 5995c GC-MS low resolution spectrometer. All compounds showed appropriate ir, ^{1}H nmr and mass spectra. Elemental analyses were carried out with Eurovector EuroEA 3000 analyzer and the results were within ± 0.40% of the theoretical values. Column chromatography on silica gel 60 (Merck 70-230 mesh) was carried out by using light petroleum ether (bp 40-70°)-ethyl acetate (7:3 v/v) or ethyl acetate-hexane (7:3 v/v) as eluent, in each case specified, and tlc was carried out by using hexane-ethyl acetate (7:3 v/v) as eluent.

General Procedure for the Preparation of Compounds **5** [5].

A mixture of the 2- or 3-chloro-4-fluoroaniline (30.0 g, 206 mmol) and potassium thiocyanate (40.0 g, 412 mmol) in glacial acetic acid (500 ml) was stirred for 5 min. Bromine (48.0 g, 300 mmol) in glacial acetic acid (500 ml) was added dropwise to this mixture, the temperature being kept below 30-35 °C throughout the addition. Stirring was continued for an additional 1 hour after the bromine addition. After cooling, the residue was removed by filtration. The filtered solution was made alkaline with 28% ammonium hydroxide and the solid precipitate was collected washed with water (3 x 50 ml). The combined water layers were made alkaline with 28% ammonium hydroxide and the resulting precipitate was combined with that previously collected.

In the following cases, alternative procedures were also used. Thus, for **5a**, the combined precipitates were dissolved in toluene, and the mixture was distilled in a Dean Stark apparatus using toluene/water azeotropic phase. The toluene layer was separated, dried (sodium sulfate) and evaporated *in vacuo*.

For **5b** and **5c**, the combined precipitates were extracted with ethyl acetate (3 x 200 ml). The organic layers were separated, dried (sodium sulfate) and evaporated *in vacuo*. The residue on tlc showed R_f 0.73 and 0.65. Column chromatography (eluent hexane-ethyl acetate 3:7) of the residue gave compounds **5b** as a pink solid, R_f 0.73 (Yield 30.0%) and compound **5c** as a white solid, R_f 0.65 (Yield 21.7%).

According to one of the two procedures, starting from 4-fluoroaniline (2 or 3 respectively)-Cl-substituted, as reported in Scheme 1 the three isomers **5a-c** were prepared, showing M.W. 202, *Anal*. Calcd. for $C_7H_4ClFN_2S$: C, 41.58; H, 1.98; N, 13.86. Each of the isomers showed *m/z* 202 ($M^{+\cdot}$ base), 175, 140. The yields and the chemical and physical properties are reported in Tables 1 and 3.

General Procedure for the Preparation of Compounds **3**.

A mixture of **5** (0.406 g, 2.00 mmol) and triethylamine (0.202 g, d = 0.726, 2.00 mmol) in dry 1,4-dioxane (20 ml) was stirred for 30 min at 50-60 °C. A solution of appropriate difluorine-benzoyl chloride (0.354 g, 2.00 mmol) in dry 1,4-dioxane (20 ml) was added dropwise, at the same temperature. The mixture was stirred for 2 hours at 50-60 °C and then poured into crushed ice. The resulting solid, so separated, was collected by filtration and washed with 1% potassium bicarbonate and water.

In the following cases, alternative procedures were also used. For **3a**, **d**, **h**, **i**, **n**, **q**, **r**, the crude precipitate was treated three times with boiling ethyl alcohol 95% and filtered; the combined filtrates, after cooling, gave crystals which were separated by filtration.

For **3b, c, e, f, g, j, k, l, m, o, p**, the crude precipitate was purified by column chromatography, eluent light petroleum ether (bp 40-70°)-ethyl acetate (7:3, v/v).

All the prepared 18 isomeric compounds **3**(**a-r**) show appropriate mass spectra. Each of the isomers showed *m/z* 342 (M^+), 141 (base), 113. The yields, the elemental analyses and the chemical and physical properties are reported in Tables 2 and 4.

Microbiology - Determination of Fungistatic Activity.

Standard strains of *Candida albicans* ATCC 10231, *Candida albicans* ATCC [6] 14053, *Candida albicans* NRRL y 869 and *Candida albicans* NRRL 12983 [7] was grown and maintained on sabouraud agar slants. The fungal strains were grown in Sabouraud dextrose agar. Five colonies were picked and suspensions were adjusted to approximately $1x10^6$ cfu/ml. They were diluted appropriately in yeast malt broth medium (National Committee Clinical Laboratory Standard USA -NCCLS 1997, m27-a) to give an inoculum of $1x10^3$ cfu/ml [8,9]. Stock solutions of the compounds were adjusted in DMSO [8]. Final concentrations ranges were for each compound from 500 to 62.5 μg/ml. A series of twofold dilutions of each agent were prepared in yeast malt broth in a 96 wells microdilution. Each well was inoculated with a final concentration of approximately 1 x 10^3 to $3x10^3$ cfu/ml as confirmed by viable counts. Microdilution trays were incubated for 48 h at 30 °C and than the growth was monitored visually. The minimum inhibitory concentration was defined as the lowest concentration required to arrest the growth of the fungi at the end of 48 h of incubation at 30 °C. Each isolate was tested at least twice in duplicate [8,9]. The results are reported in Tables 5a and b.

Acknowledgements.

This work was supported by a grant of Ministero dell'Istruzione, dell'Università e della Ricerca (M.I.U.R.) - Rome.

REFERENCES AND NOTES

* Correspondence to: Prof. Flaviano Morlacchi - Dipartimento Farmaco-chimico, Facoltà di Farmacia, Università degli Studi di Bari, Via E. Orabona 4, 70125 Bari, Italy.

[1a] Anup R. Barde, H. Barsu, Khadse and Anil S. Bobade, *Indian Drugs*, **35**, 554, (1998); [b] G. Trapani, A. Latrofa, M. Franco, D. Armenise, F. Morlacchi, G. Liso, *Arzneim. –Forsch./Drug Res.*, **44 (II)**, 8, 969, (1994).

[2] A. Alanine, A. Flohr, A. K. Miller, R. D. Norcross and C. Riemer. (F. Hoffman – La Roche A. – G. Switz.) PCT Int. Appl., WO0197786, 160 pp, (2001); *Chem. Abstr.,* **128**, 69803 (2002).

[3] S. Kerwin, L. H. Hurley, M. R. De Luca and B. M. Moore. PCT Int. Appl., WO9748694, 119 pp, (1997); *Chem. Abstr.,* **128**, 75390 (1998).

[4] C. Zagar, E. Heistracher, R. Reinhard, G. Hamprecht, M. Menges, O. Menke, P. Schafer and K. O. Westphalen, PCT Int. Appl., WO9833796, 40 pp, (1998); *Chem. Abstr.,* **129**, 148990 (1998).

[5] V. Cecchetti, A. Fravolini, R. Fringuelli, G. Mascellani, P. Pagella, M. Palmioli, G. Segre and P. Terni, *J. Med. Chem*., **30**, 3, 465, (1987).

[6] American Type Colture Collection.

[7] United States Department of Agriculture.

[8] Manual of Clinical Microbiology. Murray et al. 7th ed., ASM (USA) ed. (1999).

[9] NCCLS M27-A Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard.