SYNTHESIS, CHARACTERIZATION AND *IN VITRO* ANTIFUNGAL EVALUATION OF NOVEL BENZIMIDAZO[1,2-C]QUINAZOLINES

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SUMMARY

The synthesis of a series of new benzimidazo[1,2-*c*]quinazolines starting from 2-nitrobenzaldehyde and *o*-phenylendiamine is described. The structure elucidation of the products is based on detailed NMR analysis of experiments such as ^{1}H-COSY, NOESY, DEPT, HSQC and HMBC. These compounds showed antifungal properties only against dermatophytes. Among them, those with electron-donor substituents on the 6-phenyl ring inhibited mainly *T. rubrum and E. floccosum* with MICs between 25-250 μg/mL and *M. canis, M. gypseum and T. mentagrophytes* with MICs between 50-250 μg/mL. Structures with electron-withdrawing substituents on the phenyl ring did not show any activities up to 250 μg/ml. Methyl substituents on the benzimidazole ring seem negatively affect the antifungal behaviour of this series.

INTRODUCTION

Due to the increasing number of immunocompromised individuals, fungal infections have increased steadily in the last two decades, affecting millions of people worldwide. Opportunistic systemic mycoses are associated to high rates of deaths and skin fungal infections, although not life threatening, debilitate patients' quality of life, with the additional danger that they can spread to other areas of the body and to other individuals.

Although several drugs have been developed for the treatment of systemic an superficial mycoses, there are in fact a limited number of efficacious antifungal drugs [1,2]. Many of the currently available drugs have undesirable side-effects or are very toxic, produce recurrence, or lead to the development of resistance [3]. As a consequence, there is a real need for a next generation of antifungal agents [4].

Considering that compounds containing the quinazoline skeleton have showed varied interesting antifungal properties [5-7] and on the basis of our current project aimed at synthetic strategies for preparing heterocyclic compounds containing the quinazoline ring, we have previously synthesized and *in vitro* evaluated tetrahydropyrazolo[1,5-*c*]quinazolines for antifungal properties, showing that they do not possess any antifungal activity up to 250 μg/mL [8].

As a continuation of our previous studies, we report here the synthesis, antifungal properties and structure-activity relationships of a series of benzimidazo[1,5-c]quinazolines, not reported up to date in the literature, prepared by using 2-nitrobenzaldehyde and *o*-phenylendiamines as starting materials.

The use of 2-nitrobenzaldehyde for the design and synthesis of new quinazoline compounds is being subject of a growing interest [9,10].

In turn, substituted benzimidazoles have showed to be interesting structures from the point of view of their potential antimicrobial activity. So, interesting antifungal properties have been described for ciclopentyl and ciclohexyl-imidazoles [11], benzimidazolecarboxamides [12, 13] and sulfinyl benzimidazoles [14].

Particularly benzimidazoquinazolines have showed virucide properties [15], making these compounds interesting candidates for being submitted to antifungal evaluation. It is known that one of the strategies for finding new antifungal agents is the Comparative Design. In this approach, compounds known to be active against any microorganism different from fungi, should be tested for antifungal properties since they or their analogues could become effective against known opportunistic infections [16].

EXPERIMENTAL PROCEDURES

GENERAL

All starting material were commercially available research-grade chemicals obtained from Aldrich (Steinheim, Germany), Merck (Darmstadt, Germany) and Sigma (St Louis, MO, USA). Melting points were determined in a Büchi Melting Point Apparatus (New Castle, Delaware, England) and are uncorrected. The ^{1}H- and ^{13}C nmr spectra were run on a Bruker DPX 300 spectrometer (Bruker Karslruhe, Germany) operating at 300 MHz and 75 MHz respectively, using dimethyl sulfoxide-d_6 as solvent and tetramethylsilane as internal standard. The mass spectra were recorded on a Fisons-Platform interface APCI in MeOH and recorded on a Hewlett Packard HP Engine-5989 spectrometer (Palo Alto, California, USA) (equipped with a direct inlet probe) a operating at 70 eV. The elemental analysis have been obtained using a LECO CHNS-900 equipment (St. Joseph, Michigan, USA). The compound **3** was prepared according to reported methods.[12]

General procedure for the synthesis of 6-aryl-5,6-dihydrobenzimidazo[1,2-*c*]quinazolines (4a-l)

A solution of equimolar amounts (16 mmol) of the corresponding diamine **3** and benzaldehyde in ethanol (10 mL) was treated with acetic acid (1 mL). The mixture was stirred at room temperature for 10 minutes and then allowed in the refrigerator overnight. The resulting solid was collected and recrystallized from ethanol.

6-Phenyl-5,6-dihydrobenzimidazo[1,2-*c*]quinazoline (4a).

This compound was obtained according to general procedure as white crystals, mp 220 °C, yield 70%; ^{1}H NMR: d = 6.84 (t, 1H, H-2); 6.88 (d, 1H, H-4, ^{3}J=7.7 Hz); 7.09 (d, 1H, H-6, ^{3}J=1.3 Hz); 7.10 (t, 1H, H-9); 7.15 (d, 1H, H-8, ^{3}J=6.7 Hz); 7.18 (t, 1H, H-10); 7.26 (t, 1H, H-3); 7.32 (m, 5H, H-*o, m, p*); 7.62 (d, 1H, NH, ^{3}J=1.3 Hz); 7.67 (d, 1H, H-11, ^{3}J=7.9 Hz); 7.98 (dd, 1H, H-1, ^{3}J=7.7 Hz, ^{4}J=1.3 Hz). ^{13}C NMR: d = 68.2 (C-6); 110.9 (C-8); 112.3 (C-12b); 115.2 (C-4); 118.6 (C-2); 119.0 (C-11); 122.4 (C-9); 122.6 (C-10); 125.0 (C-1); 126.4 (2C, C-*m*); 129.1 (2C, C- *o*); 129.3 (C-*p*); 132.0 (C-3); 133.2 (C-7a); 140.8 (C-*i*); 143.5 (C-4a); 144.2 (C-11a); 147.3 (C-12a). MS (FAB): *m/z* (%) = 299 (M^++2)

Anal. Calcd. for $C_{20}H_{15}N_3$: C, 80.78; H, 5.08; N, 14.13. Found: C, 80.73; H, 5.12; N, 14.06.

6-(4-Chlorophenyl)-5,6-dihydrobenzimidazo[1,2-*c*]quinazoline (4b).

This compound was obtained according to general procedure as white crystals, mp 207 °C, yield 82%; ^{1}H NMR: d = 6.85 (t, 1H, H-2); 6.86 (d, 1H, H-4, ^{3}J=7.4 Hz); 7.13 (s, 1H, H-6); 7.14 (t, 1H, H-9); 7.20 (t, 1H, H-10); 7.23 (d, 1H, H-8, ^{3}J=7.9 Hz); 7.26 (t, 1H, H-3); 7.44 (d, 2H, H-*o*, ^{3}J=8.5 Hz); 7.40 (d, 2H, H-*m*, ^{3}J=8.5 Hz); 7.65 (s, 1H, NH); 7.67 (d, 1H, H-11, ^{3}J=7.8 Hz); 7.96 (d, 1H, H-1, ^{3}J=7.5 Hz). ^{13}C NMR: d = 67.3 (C-6); 110.8 (C-8); 112.3 (C-12b); 115.3 (C-4); 118.8 (C-2); 119.1 (C-11); 122.6 (C-9); 122.7 (C-10); 125.1 (C-1); 128.2 (2C, C-*o*); 129.2 (2C, C-*m*); 132.1 (C-3); 133.1 (C-7a); 133.9 (C-*i*); 139.8 (C-*p*); 143.2 (C-4a); 144.2 (C-11a); 147.1 (C-12a). MS (FAB): *m/z* (%) = 333 (M^++2, 100).

Anal. Calcd. for $C_{20}H_{14}ClN_3$: C, 72.40; H, 4.25; N, 12.66. Found: C, 72.38; H, 4.19; N, 12.61.

6-(4-Bromophenyl)-5,6-dihydrobenzimidazo[1,2-*c*]quinazoline (4c).

This compound was obtained according to general procedure as white crystals, mp 209 °C, yield 87%; ^{1}H NMR: d = 6.85 (t, 1H, H-2); 6.86 (d, 1H, H-4, ^{3}J=7.8 Hz); 7.14 (s, 1H, H-6); 7.16 (m, 2H, H-9, H-10); 7.19 (d, 2H, H-*o*, ^{3}J=8.4 Hz); 7.28 (d, 1H, H-8, ^{3}J=7.3 Hz); 7.29 (t, 1H, H-3); 7.50 (d, 2H, H-*m*, ^{3}J=8.4 Hz); 7.68 (d, 1H, H-11, ^{3}J=7.9 Hz); 7.69 (s, 1H, NH); 7.95 (d, 1H, H-1, ^{3}J=7.3 Hz). ^{13}C NMR: d = 67.3 (C-6); 110.8 (C-8); 112.2 (C-12b); 115.3 (C-4); 118.7 (C-2); 119.1 (C-11); 122.5 (C-*i*); 122.6 (C-9); 122.7 (C-10); 125.1 (C-1); 128.4 (2C, C-*o*); 132.1 (3C, C-*m*, C-3); 133.1 (C-7a); 140.2 (C-*p*); 143.2 (C-4a); 144.2 (C-11a); 147.1 (C-12a). MS: *m/z* (%) = 377 (M^+ +1, 100), 379 (74).

Anal. Calcd. for $C_{20}H_{14}BrN_3$: C, 63.84; H, 3.75; N, 11.17. Found: C, 63.77; H, 3.72; N, 11.22.

6-(4-Fluorophenyl)-5,6-dihydrobenzimidazo[1,2-*c*]quinazoline 4n.

This compound was obtained according to general procedure as yellow crystals, mp 204 °C, yield 85%; ^{1}H NMR: d = 6.82 (s, 1H, NH); 6.86 (t, 1H, H-2); 7.10-7.40 (m, 9H, H-arom.); 7.62 (s, 1H, H-6); 7.67 (d, 1H, H-11, ^{3}J=9.0 Hz); 7.97 (d, 1H, H-1, ^{3}J=9.0 Hz). ^{13}C NMR: d = 67.0 (C-6); 110.4 (C-8); 111.8 (C-12b); 114.8 (C-4); 118.2 (C-2); 118.7 (C-11); 122.0 (C-9); 122.2 (C-10); 124.6 (C-1); 128.0 (2C, C-*o*); 131.6 (2C, C-*m*); 132.2 (C-3); 132.7 (C-7a); 136.6 (C-*i*); 142.9 (C-4a); 143.8 (C-11a); 146.7 (C-12a); 163.8 (C-*p*). MS (EI): *m/z* (%) = 315 (M^+, 60); 220 (27); 114 (100).

Anal. Calcd. for $C_{20}H_{14}FN_3$: C, 76.18; H, 4.47; N, 13.33. Found: C, 76.31; H, 4.41; N, 13.21.

6-(4-Nitrophenyl)-5,6-dihydrobenzimidazo[1,2-*c*]quinazoline (4e).

This compound was obtained according to general procedure as white crystals, mp 252 °C, yield 91%; ^{1}H NMR: d = 6.86 (t, 1H, H-2); 6.87 (d, 1H, H-4, ^{3}J=7.5 Hz); 7.16 (t, 1H, H-9); 7.22 (t, 1H, H-10); 7.27 (t, 1H, H-3); 7.34 (s, 1H, H-6); 7.35 (d, 1H, H-8, ^{3}J=7.6 Hz); 7.44 (d, 2H, H-*o*, ^{3}J=8.4 Hz); 7.69 (d, 1H, H-11, ^{3}J=7.8 Hz); 7.81 (s, 1H, NH); 7.98 (d, 1H, H-1, ^{3}J=7.8 Hz); 8.19 (d, 2H, H-*m*, ^{3}J=7.8 Hz). ^{13}C NMR: d = 66.3 (C-6); 110.2 (C-8); 111.8 (C-12b); 114.9 (C-4); 118.5 (C-2); 118.8 (C-11); 122.4 (C-9); 122.4 (C-10); 124.1 (2C, C-*m*); 124.7 (C-1); 126.9 (2C, C- *o*); 131.8 (C-3); 132.6 (C-7a); 142.3 (C-4a); 143.7 (C-11a); 146.5 (C-12a); 147.2 (C-*i*); 147.6 (C-*p*). MS (EI): *m/z* (%) = 342 (M^+, 50), 220(50), 194(100).

Anal. Calcd. for $C_{20}H_{14}N_4O_2$: C, 70.17; H, 4.12; N, 16.37. Found: C, 70.21; H, 4.20; N, 16.31.

6-(4-Methoxyphenyl)-5,6-dihydrobenzimidazo[1,2-*c*]quinazoline (4f).

This compound was obtained according to general procedure as white crystals, mp 187 °C, yield 65%; ^{1}H NMR: d = 3.7 (s, 3H, OCH_3); 6.83 (t, 1H, H-2); 6.87 (d, 1H, H-4, ^{3}J=8.3 Hz); 6.90 (d, 2H, H-*m*, ^{3}J=8.7 Hz). 6.98 (d, 1H, H-6, ^{3}J=1.7 Hz); 7.06 (t, 1H, H-9); 7.07 (d, 1H, H-8, ^{3}J=7.6 Hz); 7.15 (m, 1H, H-10); 7.25 (d, 2H, H-*o*, ^{3}J=8.7 Hz); 7.28 (t, 1H, H-3); 7.50 (d, 1H, NH, ^{3}J=1.7 Hz); 7.65 (d, 1H, H-11, ^{3}J=7.9 Hz); 7.95 (d, 1H, H-1, ^{3}J=7.7 Hz, ^{4}J=1.2 Hz). ^{13}C NMR: d = 55.5 (OCH_3); 68.1 (C-6); 111.0 (C-8); 112.3 (C-12b); 114.5 (2C, C- *m*); 115.2 (C-4); 118.5 (C-2); 119.0 (C-11); 122.3 (C-9); 122.5 (C-10); 125.0 (C-1); 127.9 (2C, C-*o*); 132.0 (C-3); 132.4 (C-*i*); 133.2 (C-7a); 143.7 (C-4a); 144.3 (C-11a); 147.4 (C-12a); 160.1 (C-*p*). MS (FAB): *m/z* (%) = 329 (M^++2).

Anal. Calcd. for $C_{21}H_{17}N_3O$: C, 77.04; H, 5.23; N, 12.84. Found: C, 77.11; H, 5.18; N, 12.88.

6-(3,4,5-Trimethoxyphenyl)-5,6-dihydrobenzimidazo[1,2-*c*]quinazoline (4g).

This compound was obtained according to general procedure as white crystals, mp 217 °C, yield 65%; ^{1}H NMR: d = 3.61 (s, 6H, OCH_3); 3.62 (s, 3H, OCH_3); 6.70 (s, 2H, H-*o*); 6.85 (t, 1H, H-2); 6.90 (d, 1H, H-4, ^{3}J=7.8 Hz); 6.93 (d, 1H, H-6, ^{3}J=1.4 Hz); 7.10 (m, 2H, H-9, H-8); 7.17 (m, 1H, H-10); 7.27 (t, 1H, H-3); 7.53 (d, 1H, NH, ^{3}J=1.4 Hz); 7.65 (d, 1H, H-11, ^{3}J=7.8 Hz); 7.97 (dd, 1H, H-1, ^{3}J=7.7 Hz, ^{4}J=1.4 Hz). ^{13}C NMR: d = 55.76 (2C, OCH_3); 59.93 (OCH_3); 68.28 (C-6); 103.76 (2C, C-*o*); 111.94 (C-12b); 114.73 (C-4); 118.24 (C-2); 118.59 (C-11); 121.98 (C-10); 122,07 (2C, C-8, C-9); 124.52 (C-1); 131.52 (C-3); 132.86 (C-11a); 135.53 (C-*i*); 137.95 (C-*p*); 143.34 (C-4a); 143.77 (C-7a); 146.99 (C-12a); 152.93 (2C, C- *m*). MS (EI): *m/z* (%) = 387 (M^+, 34), 220 (25), 194 (100).

Anal. Calcd. for $C_{23}H_{21}N_3O_3$: C, 71.30; H, 5.46; N, 10.85. Found: C, 71.37; H, 5.38; N, 10.80.

9,10-Dimethyl-6-phenyl-5,6-dihydrobenzimidazo[1,2-*c*]quinazoline (4h).

This compound was obtained according to general procedure as white crystals, mp 230 °C (desc.), yield 63%; ^{1}H NMR: d = 2.21 (s, 3H, CH_3); 2.28 (s, 3H, CH_3); 6.81 (t, 1H, H-2); 6.83 (d, 1H, H-4, ^{3}J=8.0 Hz); 6.97 (s, 1H, H-8); 7.00 (d, 2H, H-6, ^{3}J=1.7 Hz); 7.21 (m, 3H, 2H-*m*, H-*p*); 7.31 (m, 3H, 2H-*o*, H-3); 7.43 (s, 1H, H-11); 7.55 (d, 1H, NH, ^{3}J=1.7 Hz); 7.9 (d, 1H, H-1, ^{3}J=6.8 Hz). ^{13}C NMR: d = 20.3 (CH_3); 20.5 (CH_3); 67.4 (C-6); 110.9 (C-8); 112.7 (C-12b); 115.1 (C-4); 118.5 (C-2); 119.2 (C-11); 124.7 (C-1); 126.2 (2C, C- *m*); 129.1 (3C, 2C-*o*, C-*p*); 130.9 (C-9); 131.1 (C-10, C-*p*); 131.6 (C-3); 131.8 (C-7a); 141.1 (C-*i*); 142.8 (C-4a); 143.1 (C-11a); 146.4 (C-12a). MS (FAB): *m/z* (%) = 327 (M^+ +2).

Anal. Calcd. for $C_{22}H_{19}N_3$: C, 81.20; H, 5.89; N, 12.91. Found: C, 81.23; H, 5.81; N, 12.86.

9,10-Dimethyl-6-(4-chlorophenyl)-5,6-dihydrobenzimidazo[1,2-*c*]quinazoline (4i).

This compound was obtained according to general procedure as white crystals, mp 277 °C, yield 78%; ^{1}H NMR: d = 2.23 (s, 3H, CH_3); 2.29 (s, 3H, CH_3); 6.83 (t, 2H, H-2 y H-4); 7.07 (s, 2H, H-6, H-8); 7.20 (d, 3H, H-*o* y H-3, ^{3}J=7.9 Hz); 7.39 (d, 2H, H-*m*, ^{3}J=7.9 Hz); 7.45 (s, 1H, H-11); 7.60 (s, 1H, NH); 7.93 (d, 1H, H-1, ^{3}J=7.3 Hz). ^{13}C NMRM: d = 20.3 (CH_3); 20.5 (CH_3); 67.0 (C-6); 110.8 (C-8); 112.7 (C-12b); 115.3 (C-4); 118.7 (C-2); 119.3 (C-11); 124.8 (C-1); 127.9 (2C, C-*o*); 129.1 (2C, C-*m*);, 131.1 (C-9); 131.2 (C-10); 131.7 (2C, C-3, C-7a); 133.7 (C-*p*);

140.1 (C-*i*); 142.8 (C-4a); 142.8 (C-11a); 146.3 (C-12a). MS: *m/z* (%) = 361 (M^++2, 100), 363 (34)

Anal. Calcd. for $C_{22}H_{18}N_3Cl$: C, 73.43; H, 5.04; N, 11.68. Found: C, 73.51; H, 5.11; N, 11.61.

9,10-Dimethyl-6-(4-bromophenyl)-5,6-dihydrobenzimidazo[1,2-*c*]quinazoline (4j).

This compound was obtained according to general procedure as white crystals, mp 278 °C, yield 79%; ^{1}H NMR: d = 2.23 (s, 3H, CH_3); 2.28 (s, 3H, CH_3); 6.81 (t, 1H, H-2); 6.82 (d, 1H, H-4, ^{3}J=7.6 Hz); 7.04 (d, 1H, H-6, ^{3}J=2.0 Hz); 7.06 (s, 1H, H-8); 7.11 (d, 2H, H-*o*, ^{3}J=8.5 Hz); 7.21 (t, 1H, H-3); 7.44 (s, 1H, H-11); 7.51 (d, 2H, H-*m*, ^{3}J=8.5 Hz); 7.59 (d, 1H, NH, ^{3}J=2.0 Hz); 7.91 (d, 1H, H-1, ^{3}J=7.2 Hz). ^{13}C NMR: d = 20.3 (CH_3); 20.5 (CH_3); 67.0 (C-6); 110.8 (C-8); 112.6 (C-12b); 115.2 (C-4); 118.7 (C-2); 119.3 (C-11); 122.3 (C-*i*); 124.8 (C-1); 128.2 (2C, C-*o*); 131.1 (C-9); 131.3 (C-10); 131.6 (C-7a); 131.7 (C-3); 132.1 (2C, C- *m*);, 140.5 (C-*p*); 142.7 (C-4a); 142.8 (C-11a); 146.2 (C-12a). MS: *m/z* (%) = 405 (M^+ +1, 100), 407 (68)

Anal. Calcd. for $C_{22}H_{18}N_3Br$: C, 65.36; H, 4.49; N, 10.39. Found: C, 65.42; H, 4.43; N, 10.47.

9,10-Dimethyl-6-(4-nitrophenyl)-5,6-dihydrobenzimidazo[1,2-*c*]quinazoline (4k).

This compound was obtained according to general procedure as white crystals, mp 316 °C, yield 88%; ^{1}H NMR: d = 2.24 (s, 3H, CH_3); 2.29 (s, 3H, CH_3); 6.83 (t, 1H, H-2); 6.84 (d, 1H, H-4, ^{3}J=8.1 Hz); 7.18 (s, 1H, H-8); 7.23 (t, 1H, H-3); 7.27 (d, 1H, H-6, ^{3}J=2.2 Hz); 7.37 (d, 2H, H-*o*, ^{3}J=8.7 Hz); 7.47 (s, 1H, H-11); 7.78 (d, 1H, NH, ^{3}J=2.2 Hz); 7.95 (d, 1H, H-1, ^{3}J=8.1 Hz); 8.18 (d, 2H, H-*m*, ^{3}J=8.7 Hz). ^{13}C NMR: d = 20.3 (CH_3); 20.5 (CH_3); 66.5 (C-6); 110.7 (C-8); 112.7 (C-12b); 115.4 (C-4); 118.9 (C-2); 119.4 (C-11); 124.5 (2C, C-*m*); 124.8 (C-1); 127.2 (2C, C- *o*);, 131.3 (C-7a); 131.5 (C-9); 131.6 (C-10); 131.8 (C-3); 142.3 (C-4a); 142.8 (C-11a); 146.1 (C-12a); 147.9 (2C, C-*i*, C-*p*). MS (FAB): *m/z* (%) = 372 (M^++2).

Anal. Calcd. for $C_{22}H_{18}N_4O_2$: C, 71.34; H, 4.90; N, 15.13. Found: C, 71.39; H, 4.84; N, 15.17.

9,10-Dimethyl-6-(4-methoxyphenyl)-5,6-dihydrobenzimidazo[1,2-*c*]quinazoline (4l).

This compound was obtained according to general procedure as white crystals, mp 229 °C, yield 55%; ^{1}H NMR: d = 2.21 (s, 3H, CH_3); 2.27 (s, 3H, CH_3); 3.68 (s, 3H, OCH_3); 6.82 (t, 2H, H-2); 6.84 (d, 1H, H-4, ^{3}J=7.5 Hz); 6.87 (d, 2H, H-*m*, ^{3}J=8.7 Hz). 6.95 (s, 2H, H-6, H-8); 7.15 (d, 2H, H-*o*, ^{3}J=8.7 Hz); 7.21 (t, 1H, H-3); 7.43 (s, 1H, H-11); 7.51 (s, 1H, NH); 7.91 (d, 1H, H-1, ^{3}J=7.7 Hz). ^{13}C NMR: d = 20.3 (CH_3); 20.6 (CH_3); 55.5 (OCH_3); 67.6 (C-6); 111.0 (C-8); 112.6 (C-12b); 114.4 (2C, C- *m*); 115.1 (C-4); 118.4 (C-2); 119.1 (C-11); 124.7 (C-1); 127.5 (2C, C-*o*); 130.8 (C-9); 131.0 (C-10); 131.6 (C-3); 131.7 (C-7a); 133.2 (C-i); 142.8 (C-11a); 143.3 (C-4a); 146.5 (C-12a); 159.9 (C-*p*). MS (FAB): *m/z* (%) = 357 (M^+ +2)

Anal. Calcd. for $C_{23}H_{21}N_3$: C, 77.72; H, 5.96; N, 11.82. Found: C, 77.67; H, 5.89; N, 11.77.

Biological evaluation

Microorganisms and media

The microorganisms used for the fungistatic evaluation were purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA) or were clinical isolates kindly provided by Centro de Referencia Micológica (C, CEREMIC), Facultad de Ciencias Bioquímicas y Farmacéuticas, Suipacha 531-(2000)-Rosario, Argentina. *Candida albicans* ATCC 10231, *Saccharomyces cerevisiae* ATCC 9763, *Cryptococcus neoformans* ATCC 32264, *Aspergillus flavus* ATCC 9170, *Aspergillus fumigatus* ATCC 26934, *Aspergillus niger* ATCC 9029, *Trichophyton mentagrophytes* ATCC 9972. *Microsporum canis* C 112, *Trichophyton rubrum* C 113, *Epidermophyton floccosum* C 114, *Microsporum gypseum* C 115, and *Candida tropicalis* C131. The strains were maintained on slopes of Sabouraud-dextrose agar (SDA, Oxoid) and subcultured every 15 days to prevent pleomorphic transformations. Spore suspensions were obtained according to reported procedures [17] and adjusted to 10^6 spores/cells with colony forming ability/mL.

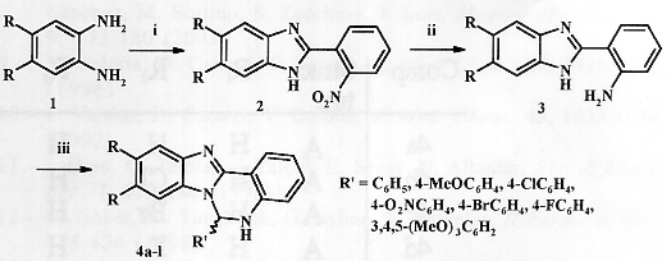
Antifungal assays

The antifungal activity of benzimidazo[1,2-*c*]quinazolines was evaluated with the agar dilution method by using Sabouraud-chloramphenicol agar for both yeast and dermatophyte species as previously described [18,19]. Stock solutions of compounds (10 mg/mL in DMSO) were diluted to give serial two-fold dilutions that were added to each medium resulting in concentrations ranging from 0.10 to 250 μg/mL. The minimum inhibitory concentration (MIC) for each compound was defined as the lowest concentration that produces no visible fungal growth after the incubation time. Amphotericin B (Sigma Chem. Co.), Ketoconazole (Janssen Pharmaceutica) and Terbinafine (Novartis) were used as positive controls.

RESULTS AND DISCUSSION

CHEMISTRY

The synthesis of benzimidazo[1,2-*c*]quinazolines **4** was performed from 2-(2-aminophenyl)benzimidazole **3**, following Scheme 1. The starting compound was readily prepared in high yield by reduction of 2-(2-nitrophenyl)benzimidazole **2**. The benzimidazo [1,2-*c*]quinazolines were obtained by reaction with aldehydes in ethanol/acetic acid mixture.



i) 2-$O_2NC_6H_4CHO$, EtOH/HCl; ii) Ni-Raney/MeOH, $N_2H_4.H_2O$;
iii) R'CHO/EtOH,AcOH

Scheme 1. Synthesis of benzimidazo[1,2-*c*]quinazolines using *o*-phenylendiamines as starting materials

The formation of **4** as the unique product of reaction was confirmed by spectroscopic analyses. The IR spectra of compounds **4** measured in KBr pellets showed a band of the elongation vibrations corresponding to the C=N group at 1620-1640 cm^{-1} and another band at 3420-3450 cm^{-1} attributed to the NH-group.

Together with the aromatic proton signals at 7.16-7.57 ppm, a broad singlet at 7.5-7.8 ppm corresponding to the NH group and a hardly resolved doublet at 6.9-7.4 of the proton in the 6-position of the quinazoline ring (^{3}J = 1.4-1,7 Hz) were observed in the ^{1}H-NMR spectra of compounds **4a-l**.

DEPT experiments in the ^{13}C-NMR spectra allowed to assign the signals belonging to quaternary, tertiary and primary carbon atoms for compounds **4a-l** and corroborated the proposed structures. The full assignment of the signals is supported by 2D COSY, HMQC and HMBC NMR spectra.

The presence of a molecular ion at m/z 299 (M^++2) in the mass spectrum was consistent with the structure **4a**. All spectra described in this paper are available from authors on request.

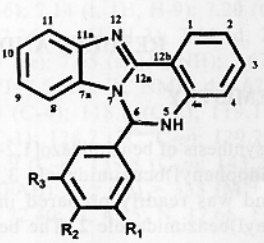
ANTIFUNGAL ASSAYS

Compounds **4** were assayed for antifungal properties against a panel of human opportunistic pathogenic yeasts, filamentous fungi as well as dermatophytes with the agar dilution method. To carry out the antifungal evaluation, concentrations up to 250 μg/mL were incorporated to growth media according to reported procedures [18, 19].

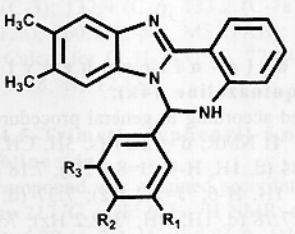
Three type of antifungal drugs in clinical use were incorporated to the assays as positive controls. They have been chosen considering that each one acts through a different mechanism of action and in addition, are commonly used for treating infections provoked for fungal species included in the test panel. Amphotericin B (which acts by binding to ergosterol forming pores in the fungal membrane) [20, 21] is also the drug of choice for systemic mycoses produced by *Candida* and other yeasts many times resistant to azoles [22, 23, 24]. Terbinafine acts by inhibiting the enzyme squalene epoxidase, which catalyzes the epoxidation of squalene in the biosynthesis of ergosterol. It is the drug of choice for the treatment of dermatophytoses [25, 26]. Retocoazole, which Ketoconazole, which possesses the typical azole ring in its structure (ring that is also present in benzimidazoles tested here) acts by inhibiting the biosynthesis of ergosterol trough the inhibition of 14-demethylase. Azoles are intensely used for the treatment of all mycoses and many fungi have developed resistance to them [27].

Results showed than none of the compounds tested was active neither against the yeasts *C. albicans*, *S. cerevisiae* or *C. neoformans* nor against the filamentous fungi *A. niger*, *A. fumigatus* or *A. flavus* (results not shown). In contrast, some compounds of the series showed antifungal effects against dermatophytes (Table 1).

Table 1: MICs values (μg/mL) of benzimidazole[1,2-*c*]quinazolines acting against human opportunistic pathogenic fungi.



A



B

Comp	Structure	R_1	R_2	R_3	*M.c.*	*M.g.*	*T.r.*	*T.m*	*E.f.*
4a	A	H	H	H	125	125	125	125	100
4b	A	H	Cl	H	>250	>250	>250	>250	>250
4c	A	H	Br	H	>250	>250	>250	>250	>250
4d	A	H	F	H	>250	>250	>250	>250	>250
4e	A	H	NO_2	H	>250	>250	>250	>250	>250
4f	A	H	OCH_3	H	250	250	25	50	25
4g	A	OCH_3	OCH_3	OCH_3	250	250	25	50	25
4h	B	H	H	H	>250	>250	>250	>250	>250
4i	B	H	Cl	H	>250	>250	>250	>250	>250
4j	B	H	Br	H	>250	>250	>250	>250	>250
4k	B	H	NO_2	H	>250	>250	>250	>250	>250
4l	B	H	OCH_3	H	125	>250	125	125	25
Amp					6.25	6.25	25	6.25	0.30
Ket					15	6.25	15	12.5	25
Terb					0.01	0.04	0.01	0.04	0.004

M.c.: *Microsporum canis* C 112. *M.g.*: *Microsporum gypseum* C 115, T.*r.*: *Trichophyton rubrum* C 113, *T.m.*: *Trichophyton mentagrophytes* ATCC 9972, *E.f.*: *Epidermophyton floccosum* C 114. Amp= Amphotericin B. Ket=Ketoconazole.

Terb=Terbinafine.

Since dermatophytes are a group of fungi which characteristically infect the keratinized areas of the body and dermatomycoses are very difficult to eradicate, it is very interesting to note that these benzimidazo[1,2-*c*]quinazolines showed activity against dermatophytes and not against another type of fungi.

To evaluate the structure-activity relationships, the effect of electron-donor and electron-withdrawing substituents on the 6-phenyl ring of structures possessing A) a non-substituted benzimidazole (compounds **4a-g**); B) a 9,10-dimethyl substituted benzimidazole (compounds **4h-l**) were analyzed.

Among compounds with non-substituted benzimidazole (type A), only structures **4a, 4f, 4g** showed significant activity against all the dermatophytes tested. Non-substituted compound **4a** showed a moderate activity with MICs between 100-125 µg/mL. The introduction of electron-withdrawing substituents such as Cl, Br, F and NO_2 on the 6-phenyl ring rendered compounds **4b, 4c, 4d** and **4e** respectively, all of them devoid of antifungal activity. In contrast, the replacement of the *para*-H atom in structure **4a** by a donor substituent such as OCH_3 group ($\longrightarrow$ **4f**), enhanced the activity 2.5-5 times in *T. mentagrophytes, E. floccosum* and *T. rubrum.* The addition of two extra OCH_3 groups (**4f** $\longrightarrow$ **4g**) does not improve the activity, both compounds showing the same MICs against all dermatophytes tested.

Regarding the influence of methyl substituents on both 9 and 10-positions of the benzimidazole ring (compounds type B, **4h-4l**), results showed that this structural change produced a decrease in the antifungal activity in compounds without substituent or with donor groups (compare activities of **4h**/**4a**; **4l**/**4f**), and do not modify the lack of activity of structures with electron-withdrawing substituents (compare activities of **4b**/**4i**; **4c**/**4j** or **4e**/**4k**) particularly against *T. rubrum, T. mentagrophytes* and *E. floccosum.* Figure 1 illustrates this effect for *T. rubrum.*

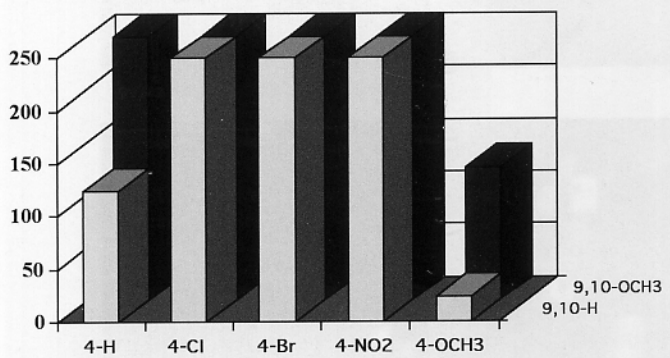


Figure 1: Comparative MICs in µg/ml of (9,10-Me-) vs. (9,10-H) -6-(4-R-phenyl)benzimidazo[1,2-c]quinazolines against *Trichophyton rubrum.* R= H, Cl, Br, NO_2, OCH_3

It is interesting to note that antifungal benzimidazole quinazolines showed activities only against dermatophytes and among them particularly against *T. rubrum* and *E. floccosum. T. rubrum* is the cause of approximately 80-93% of chronic and recurrent dermatophyte infections in human beings [28] and *E. floccosum* along with *T. rubrum* are the most frequent ethiological agents of *tinea cruris*, infection that affect the perianal and perineal areas of adult men producing bilateral erythematous lesions which are highly contagious. In addition *E. floccosum* produces arthroconidia which survive for a longer time than those of other dermatophytes, therefore constituting an environmental source of contagion, which could lead to recurrent outbreaks of dermatophytosis in individuals and in institutions [28].

Although benzimidazole compounds presented here showed lower activities than the available antifungal drugs, it is important to take in account that resistance to all antifungal agents in clinical use is growing at an alarming rate due in part to the treatment of the chronic different *tineas* with the same broad spectrum antifungal agent. So, narrow spectrum compound are intensely needed for treating dermatomycoses where the ethiological agent is known [29]. Therefore, we hope that the study of the antifungal properties of new benzimidazole-quinazoline compounds open new avenues for the development of another antifungals which, in the future, could be useful for treating the obstinate dermal fungal infections.

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REFERENCES

1.- D. Li, R. Calderone, Antifungal drugs, targets and target discovery; in: G. San-Blas, R. Calderone. (Eds), *Pathogenic fungi, host interaction and emerging strategies for control*, Caister Academic Press, Norfolk. 335-355 (2004)

2.- C. Selitrennikoff, *Antifungal drugs*: (1,3)-β-glucan-synthase inhibitors, Springer-Verlag, Heidelberg. 1–12 (1995)

3.- T. White, K. Marr, R. Bowden, *Clin. Microbiol. Rev.,* **11**, 382-402 (1998)

4.- D. Denning, *Drug Plus Intern.*, **3,** 11-13 (2004)

5.- J. H. Chan, J. S. Hong, L. F. Kuyper, D. P. Baccanari, S. S. Joyner, R. L. Tansik, C. M. Boytos, S. K. Rudolph, *J. Med. Chem.,* **38**, 3608-3616 (1995)

6.- R. Castaldo, D. Gump, J. Mc Cormack, *Antimicrob. Agents Chemother.*, **15**, 81-86 (1979)

7.- J. Bartroli, E. Turmo, M. Algueró, E. Boncompte, M. Vericat, L. Conte, J. Ramis, M. Merlos, J. García-Rafanell, J. Forn, *J. Med. Chem.,* **41**, 1869–1882. (1998)

8.- B. Insuasty, H. Torres, J. Quiroga, R. Abonía, M. Nogueras, A. Sánchez, M. Sortino, S. Zacchino, J. Low, *Heterocyclic Commun.,* **9**, 153-160 (2003)

9.- V. Colotta, D. Catarzi, F. Varano, *J. Med. Chem.,* **39**, 2915-2921 (1996)

10.- F. Varano, D. Catarzi, V. Colotta, *J. Med. Chem.,* **45**, 1035-1044 (2002)

11.- i. Ören, Ö. Temiz, . i Yalçin, E. Sener, N. Altanlar, *Eur. J. Pharm. Sci.*, **7**, 153-160 (1998)

12.- H. Göker, M. Tunçbilek, G. Ayhan, N. Altanlar, *Il Farmaco*, **53**, 415-420 (1998)

13.- G. Ayhan, M. Tunçbilek, N. Altanlar, H. Göker, *IL Farmaco,* **54**, 562-565 (1999)

14.- H. Küçükbay, R. Durmaz, E. Orhan, S. Günal, *Eur. J. Med. Chem.,* **37**, 409-418 (2002)

15.- B. Fernández, J. Castellano, M. Redondo, Eur. Pat. Appl., EP 331,093 (Cl. C071D487/04) (1989)

16.- S. Ablordeppey, P. Fan, J. Ablordeppey, L. Mardenborough, *Curr. Med. Chem.*, **6**, 1151–1195 (1999)

17.- L. Wright, E. Scott, S. Gorman, *J. Antimicrob. Chemother.,* **12**, 317-327 (1983)

18.- S. Zacchino, S. López, G. Pezzenati, R. Furlán, C. Santecchia, L. Muñoz, F. Giannini, A. Rodríguez, R. Enriz, *J. Nat. Prod.,* **63**, 1353-1357 (1999)

19.- L. Vargas, M.V. Castelli, V. Kouznetsov, J. Urbina, S. López, M. Sortino, R. Enriz, J.C. Ribas, S. Zacchino, *Bioorg. Med. Chem.,* **11,** 1531-1550 (2003)

20.- I. Gruda, P. Nadeau, J. Brajtburg, G. Medoff, *Biochim. Biophys. Acta,* **602**, 260-268 (1980)

21.- N. Georgepapadakou, B. Dix, S. Smith, J. Feundenberger, P. Funke, *Antimicrob. Agents Chemother.*, **31**, 46-51 (1988)

22.- D. Denning, *Drug Plus Intern.*, **3**, 11-13 (2004)

23.- B. Di Domenico, *Curr. Opin. Microbiol.,* **2**, 509-515 (1999)

24.- D. Kontoyannis, E.Mantadakis, G. Samonis, *J. Hospital Infection*, **53**, 243-258 (2003)

25.- I. Weitzmann, R.Summerbell, *Clin. Microbiol. Rev.,* 8, 240-259 (1995)
26.- A. Oehlschlager, E. Czyzewska, Rationally designed inhibitors of sterol biosynthesis; in: Sutcliffe and Georgopapadakou (Eds), *Emerging targets in Antibacterial and Antifungal Chemotherapy*, Chapman and Hall, New York, 437-475 (1992)
27.- S. Kelly, D. Lamb, A. Corran, B. Baldwin, D. Kelly, *Biochem. Biophys. Res. Commun.* **207**, 910-915 (1995)
28.- Weitzman, R. Summerbell, *Clin. Microb. Rev.,* **8**, 240-259 (1995)
29.- M. Ghannoum, L. Rice, *Clin. Microb. Rev.,* **12**, 501-517 (1999)