Monatshefte für Chemie - Chemical Monthly
https://doi.org/10.1007/s00706-020-02575-9

ORIGINAL PAPER

Eco-friendly synthesis and antifungal evaluation of *N*-substituted benzimidazoles

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Received: 23 October 2019 / Accepted: 12 March 2020
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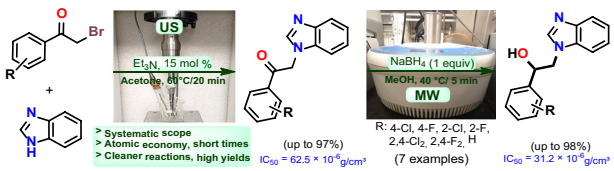
Abstract

A convenient synthesis of *N*-phenacylbenzimidazoles in high yields (90–95%) by the *N*-alkylation reaction of 1*H*-benzimidazole with phenacyl bromides is provided. The carbonyl group reduction in the products offered the respective *N*-(2-aryl-2-hydroxyethyl)benzimidazoles in yields up to 97%. In the optimization of reaction conditions for preparing these *N*-substituted benzimidazoles (ketones and alcohols), a comparative study between eco-friendly methods (microwave and ultrasound) and conventional heating is described. These antifungal azoles analogs were tested for in vitro antifungal activity against *Candida albicans* and *Cryptococcus neoformans*, where the alcohols chlorine substituted (4-Cl and 2,4-Cl_2) showed the best activity ($MIC_{50} = 31.2 \times 10^{-6}$ g/cm^3).

Graphic abstract



Keywords Alkylation · Antifungal activity · Benzimidazoles · Green synthesis · Microwave · Ultrasound

Introduction

Green experimental processes have become the major drive for synthetic organic chemists to develop their research in chemicals of biological or industrial impact [1, 2]. Azaheterocycles are a crucial group of compounds that have a wide range of uses in both medicinal chemistry and materials science [3, 4]. Among these compounds, azoles play an important role as antifungal [5], as *N*-donor ligands to form coordination complexes [6], and as part of organic fluorophores or whitening agent dyes [4, 7]. In fact, some imidazole derivatives such as clotrimazole, miconazole, and ketoconazole were the first developed antifungals that are used for the usage of infections caused by dermatophytes, yeasts, and other fungi [8, 9]. Later, a first group of 1,2,4-triazole-based antifungal drugs such as fluconazole and itraconazole appeared in the market, which were followed by new generations, including voriconazole (Fig. 1) [10]. These triazoles not only have an azole ring, but also the privileged 2-haloaryl-2-hydroxyethyl moiety at position 1 (bold fragment in Fig. 1 and Scheme 1), which can improve the biological properties by increased flexibility, solubility, absorption, and transport of drugs (specific properties of antifungal and antibacterial) [11–13].

It is noteworthy that a plethora of synthetic approaches of antifungals' active principles does not have procedures simple and environmentally benign. Likewise, there are many

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00706-020-02575-9) contains supplementary material, which is available to authorized users.

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Published online: 28 April 2020

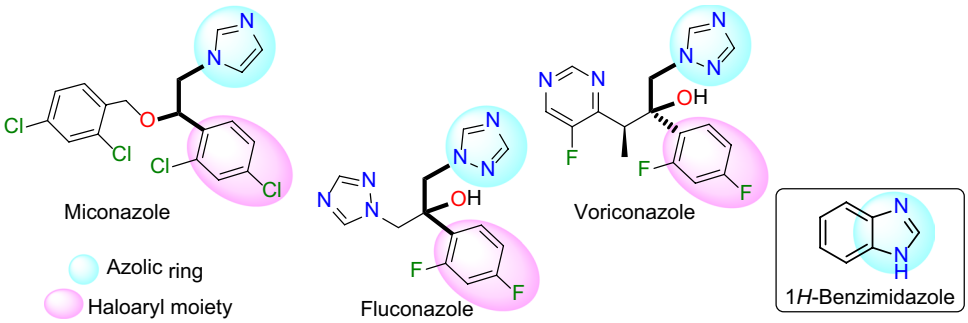
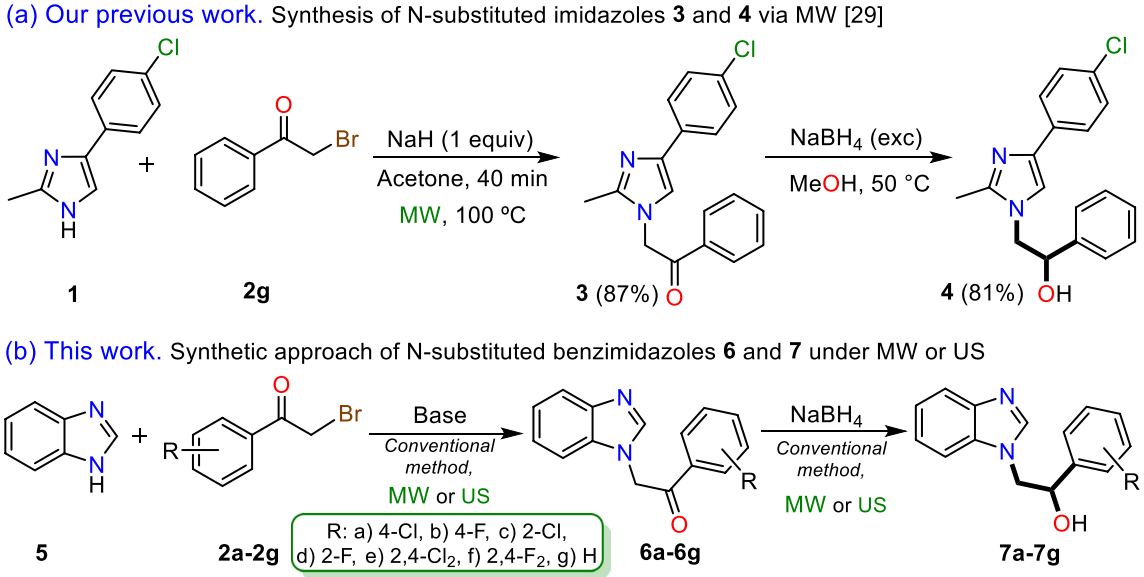


Fig. 1 Structure of some azole drugs (containing the privileged 2-aryl-2-hydroxyethyl moiety) and 1*H*-benzimidazole

Scheme 1



antifungal agents having different aza-heterocyclic moieties. For example, 1,3,5-thiadiazine-2-thiones have been obtaining with carbon disulfide in dichloromethane, both reagent and solvent being toxic [14]. Other examples are triazole and pyrimidine derivatives, where long reaction times, expensive catalysts, and prolonged purification processes decrease the synthetic efficiency and effectivity [15, 16]. On the other hand, azoles having benzimidazole scaffold have shown pharmacological value as antihypertensive, anticancer, anti-inflammatory, and anticoagulant agents [17–23], as well as for their antimicrobial, antiparasitic, antioxidant, and antiviral activities [24–26]. Likewise, diverse benzimidazoles has shown important activity antifungal [27] because of the electronic properties of its azolic pharmacophore (they have π-conjugation and N atoms), which can increase the inhibition in fungus by non-covalent interactions such as Van der Waals forces, π stacking and hydrogen bond [28, 29]. Therefore, it is crucial to develop an efficient and green approach to incorporate the moiety 2-haloaryl-2-hydroxyethyl in the benzimidazole ring.

Benzimidazole derivatives are often obtained by cyclization between *o*-phenylenediamines and different electrophiles or by *N*-alkylation/arylation of NH-benzimidazoles; alternatively, the substituted benzimidazoles can be obtained by aromatic substitution reactions on the C atoms of the ring [30–37]. These approaches and most other syntheses frequently proceed under conventional methods involving the use of additives or catalysts, waste-to-energy, long reaction times, and tedious purification steps; thus, leading to high costs and generation of a high percentage of chemical waste. Research on greener method such as ultrasound (US) and microwave (MW) irradiation is crucial to reduce the negative impact of traditional methods [38, 39]. Both tools offer a useful and facile pathway for a plethora of syntheses with diverse advantages, such as short reaction times, eco-friendly access (small volume of solvents, fewer byproducts, atomic economy, and energy efficiency), many times improved yields, and reactions work using poorly soluble reagents [1, 2, 38–41]. Therefore, the aim of this work is to obtain, in a fast and efficient way, *N*-substituted

benzimidazoles by *N*-alkylation and reduction reactions, with the aid of green methods (US and/or MW) and then, to test them against clinical important fungi (Scheme 1b).

Regarding the *N*-alkylation on NH-azoles with phenacyl halides, the reaction has been carried out using an excess of base (Et_3N, K_2CO_3, NaH, etc.), long reaction times (up to 24 h) and in diverse solvents (acetone, dioxane, acetonitrile, or dimethylformamide) at temperatures up to 90 °C [42–45]. Though there is a previous report using MW for this type of reaction in just 10 min at 170 °C [46], results with US were not found. The high temperature used is a result of lower reactivity of the phenacyl halogenures versus alkyl halides, since the substrate have a carbonyl group adjacent to the electrophilic reaction center (Cα as a carbanion source) [42–46]. To get the privileged 2-aryl-2-hydroxyethyl moiety at position 1 of the azole ring, it is necessary to carry out the reduction of the carbonyl group of the respective *N*-phenacylazole, often performed with sodium borohydride ($NaBH_4$) in methanol. For this process, the use of at least 3 equiv of $NaBH_4$ with reaction times of up to 6 h has been reported [47–49], but methods under MW and US have not been described, possibly due to the simplicity and efficiency of the known protocols [47–49].

Our lab recently reported a MW-assisted N-alkylation of the NH-imidazole **1** with phenacyl bromide (**2g**) and 1 equiv of NaH in acetone to form the *N*-phenacylimidazole **3** (Scheme 1a) [50]. However, this was a single example to obtain an imidazole with two different aryl groups, since we have obtained other four imidazole derivatives with the same aryl group by a MW-assisted pseudo-tricomponent reaction of acetamidine with 2 equiv of *α*-bromoketone. The reduction reaction of the carbonyl group of **3** (and other four derivatives) to form the alcohol **4** using a good excess of $NaBH_4$ at 50 °C for 3 h was also carried out by us [50]. The compounds obtained in our previous work (ketones and alcohols) were tested for antifungal properties against *Candida* (*C.*) *albicans* and *Cryptococcus* (*C.*) *neoformans*. All compounds showed good activities against *C. neoformans* but ketones displayed better activities ($MIC_{50} = 15.6–62.5 \times 10^{-6}$ g/cm^3) than alcohols ($MIC_{50} = 31.2–250 \times 10^{-6}$ g/cm^3). It is important to note that the dihalogenated compounds displayed the lowest MIC_{50} values (15.6×10^{-6} g/cm^3) [50].

Inspired by our previous results along with our interest in the development of novel and greener protocols to obtain N-heterocycles of biological and photophysical interest [50–54], we proposed to carry a MW and/or US-assisted synthesis of *N*-substituted benzimidazoles **6** and **7**. These azoles have not only the benzimidazole pharmacophore, but also the privileged 2-haloaryl-2-hydroxyethyl moiety at position 1 (Scheme 1b). It is important to mention that very few examples of the synthesis of such compounds were reported in the literature, and they generally included some operational drawbacks such as excessive use of reagents or additives, long reaction times, expensive or tedious procedures, etc. [11, 55, 56]. Likewise and to the best of our knowledge, similar compounds to **6** and **7** have shown pharmacological interest as antifungal and anticonvulsant activity [57, 58], but with the specific structure of haloaryl-substituted benzimidazole, does not exist studies with fungi strains *C. albicans* and *C. neoformans*. In this way, **6a**–**6g** and **7a**–**7g** were tested for antifungal activity against two clinically important fungal species, *C. albicans* and *C. neoformans*. *C. albicans* is the fourth leading cause of nosocomial bloodstream infection (BSI) in intensive care units, causing fatal invasive candidiasis in a high percentage of patients [59, 60]. In turn, *C. neoformans* is an opportunistic fungus that causes cryptococcal meningitis that kills most AIDS affected patients worldwide [61]. Since the treatment of these patients is based on amphotericin B and fluocytosine, which were discovered nearly 50 years ago, new antifungal chemical structures for treating cryptococcoses are highly welcome [62].

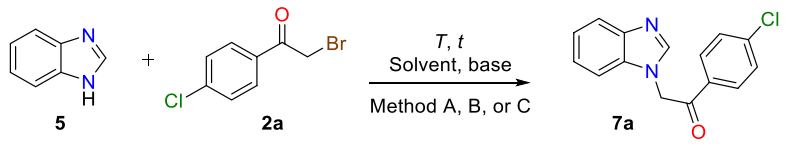
Results and discussion

Synthesis

Given our recent results regarding the synthesis of the imidazolic ketone **3** and alcohol derivative **4**, we sought to expand the scope of reactions using 1*H*-benzimidazole (**5**) and different substrates **2a**–**2g** (Scheme 1) [50]. Likewise, we want to establish MW or US-assisted methods for an improved synthesis of *N*-substituted benzimidazoles. In this respect, we tested the type of energy method to induce the synthesis, moving from conventional heating to MW and US irradiation in the model reaction with 4-chlorophenacyl bromide (**2a**) to optimize both the N-phenacylation of **5** and the carbonyl group reduction of **6** (Tables 1, 2). Initially, we studied the N-phenacylation reaction under reflux in acetone using inorganic bases, according to previous results of similar reactions [50, 63, 64]. As expected, the *N*-(4-chlorophenacyl) derivative **6a** was obtained, but the reaction proceeded in poor yields and the byproduct formation (Table 1, entries 1, 2). When the reaction was carried out using triethylamine (Et_3N) as an organic base to ensure greater solubility in the reaction medium, the yield increases up to 60% (Table 1, entries 3–5). It is possible than the deficit of this reaction is because of the amphoteric character of the azole leading to the formation of salts, so that an excess of substrate and base would improve the yield [65]; thus, the reaction by conventional heating is optimized using an excess of Et_3N and of **2a** (Table 1, entry 6).

We continue our exploratory study using MW irradiation under the same conditions reported in our previous work (NaH and 100 °C in acetone [50]), but the reaction

Table 1 Optimization of reaction conditions for synthesis of *N*-(4-chlorophenacyl)benzimidazole (**6a**)



Entry	**2a**/equiv	Base (equiv)	*T*/°C	*t*/min	Yield/%
1	1	K_2CO_3 (1)	Reflux	120	22
2	1	NaH (1)	Reflux	120	18
3	1	Et_3N (1)	Reflux	90	50
4	1	Et_3N (1)	Reflux	120	45
5	1	Et_3N (2)	Reflux	90	60
6	2	Et_3N (1.5)	Reflux	90	74[MA]
7	2	NaH (1.5)	100[a]	40	39
8	2	NaH (1.5)	120[a]	40	–
9	2	Et_3N (1.5)	100[a]	20	64
10	2	Et_3N (1.5)	100[a]	30	85[MB]
11	2	Et_3N (1.5)	55[b]	20	91
12	1	Et_3N (1)	55[b]	40	65
13	1.5	Et_3N (1)	55[b]	10	73
14	1.5	Et_3N (1)	40[b]	40	56
15	1.5	–	55[b]	40	45
16	1.5	Et_3N (cat.)[c]	55[b]	20	90[MC]
17[d]	1.5	Et_3N (cat.)[c]	80[b]	60	46
18[e]	1.5	Et_3N (cat.)[c]	60[b]	60	81

Reaction conditions: 1*H*-benzimidazole (**5**, 0.2 mmol) in acetone. Reaction by heating conventional in 2 cm^3 of solvent (Method A)

[a]Run in 10 cm^3 sealed tube under MW in 0.4 cm^3 of solvent (Method B)

[b]Run in a two-neck flask of 25 cm^3 by US in 2.0 cm^3 of solvent (Method C)

[c]Reaction with ~ 10 mol% of base

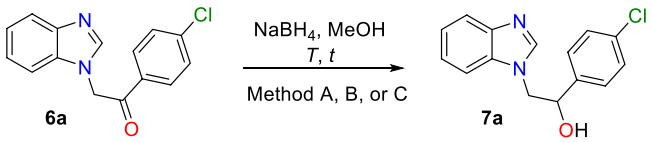
[d]In 2.0 cm^3 of water or [e]2.0 cm^3 of a water:acetone mixture (1:1 v/v)

proceeded with poor yield, occurring the decomposition of reaction mixture at a higher temperature (Table 1, entries 7, 8). The reaction with Et_3N at 100 °C for 20 min led to the desired product **6a** in good yield, but when we performed the test for 30 min, the reaction afforded the highest yield (Table 1, entries 9, 10). These results can be due to more focused heating in MW, with the advantage that temperatures above the boiling point of solvents can be used to improve yields (thermal MW effect) [63].

To finish the study of the N-phenacylation of **5**, we explored the reaction using an US probe (750 W and 20×10^3 Hz) due to the cavitation effect widely study in synthesis [2, 38, 40]. Fortunately, we found that the reaction proceeds in high yield at 55 °C for 20 min under conditions similar to those optimized reactions by both conventional heating and MW (Table 1, entries 6 and 10 vs. 11). Thus, we diminish the conditions (time, equiv of reagents and/or base) to achieve a greater atomic economy and energy efficiency versus the other methods (Table 1, entries 12–16). We found that an excellent yield is achieved with only 1.5 equiv of **2a** and catalytic quantities of base at 55 °C for 20 min (Table 1, entry 16). It is possible that under these reaction conditions, collateral reactions between the released HBr with solvent molecules are favored (aldol condensation products) [66]. Consequently, a catalytic amount of base (Et_3N) is sufficient to successfully start and complete the reaction, and this favors greater control in the process avoiding the formation of benzimidazolium salts [67].

The reaction in water was studied to find an even more eco-friendly approach, but only in a water: acetone mixture (1:1 v/v), high yield was obtained (Table 1, entries 17 vs. 18). With the best-optimized reaction conditions in hand (Table 1, entry 16), we then examined the scope of this reaction with diverse substrates. The US-assisted reaction between 1.0×10^{-3} mol of **5** with a wide range of phenacyl bromides **2a**–**2g** (1.5×10^{-3} mol) and ~ 10 mol% of Et_3N gave *N*-phenacyl derivatives **6a**–**6g** in high yields. Almost no loss of efficiency was observed when the substrates were

Table 2 Optimization of reaction conditions for synthesis of *N*-[2-(4-chlorophenyl)-2-hydroxyethyl]benzimidazole (**7a**)



Entry	$NaBH_4$/equiv	*T*/°C	*t*/min	Yield/%
1	2	50	240	35
2	4	50	240	80[MA]
3	4	25	240	Traces
4	2	50[b]	10	96
5	1	40[b]	5	97[MB]
6	1	30[b]	5	40
7[d]	1	50[b]	10	45
8[e]	1	40[b]	10	95
9[f]	3	40[b]	10	96
10	2	40[c]	10	91
11	1	40[c]	10	93[MB]
12[g]	1	40[c]	10	51
13[g]	2	40[c]	10	59
14[g]	2	40[c]	20	78
15[g]	2	40[c]	30	93

Reaction conditions: *N*-(4-chlorophenacyl)benzimidazole (**6a**, 0.4×10^{-3} mol) in methanol under heating conventional in 5.0 cm^3 of solvent (Method A)

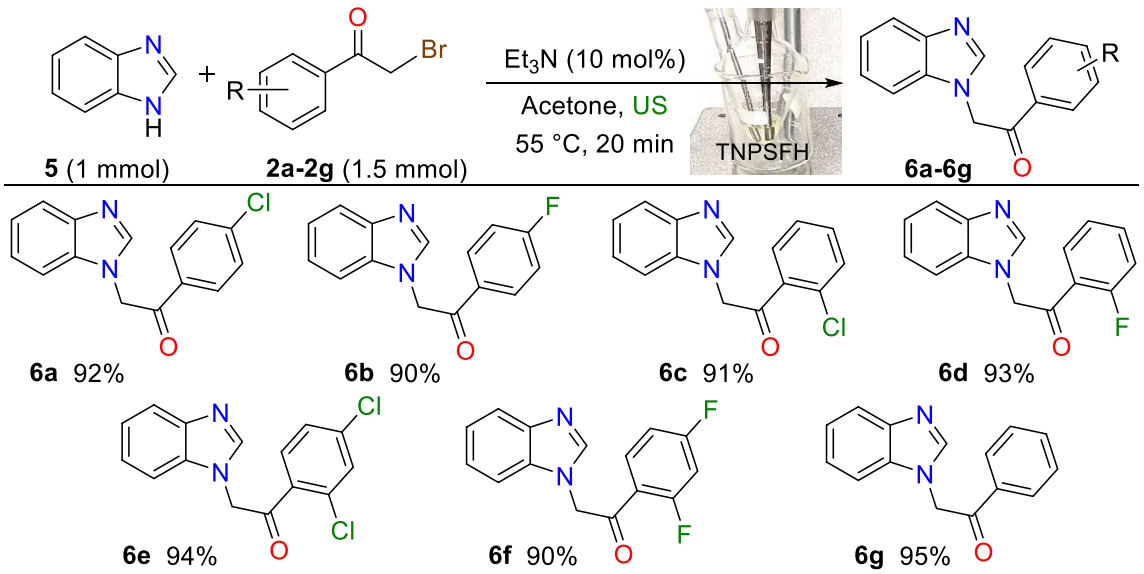
[a]Run in 10 cm^3 sealed tube under MW in 1.0 cm^3 of solvent (Method B)

[b]Run in a two-neck flask of 25 cm^3 by US in 5.0 cm^3 of solvent (Method C). MB in [c]H_2O, [d]MeOH:H_2O (1:1 v/v) or [e]MeOH:H_2O (1:9 v/v).[f] MC in H_2O

tested, which indicated that the electronic demands of the substituents had little influence on the reactivity (Scheme 2).

It is important to clarify that the reaction vessel affects the time, temperature, and yield, where the best results were achieved with a glass two necks pear shape flask heart

Scheme 2



(TNPSFH), while it was evident that the reaction was not complete using other flasks under the best-found conditions. Apparently, the convex cavity of TNPSFH accomplished to a greater effect of ultrasound wave via a better contact of the reaction mixture with the probe (Scheme 2). Once compounds **6a**–**6g** were obtained, we wanted to develop an operationally simple method to convert these ketones in the corresponding alcohols **7a**–**7g** by protocols described in the literature ($NaBH_4$ in methanol) [49, 50] but under an US or MW-assisted synthesis to know the advantages of these ways versus conventional heating. Initially, we observed that in the model-reaction with **6a** under conventional heating at 50 °C, an excess of $NaBH_4$ were needed to give the expected alcohols **7a** in high yield and by TLC, we noted that the reaction at room temperature proceeded with very low conversion (Table 2, entries 1–3). Therefore, the reaction by this method is optimized using 4 equiv of $NaBH_4$ at 50 °C for 4 h.

We continue our research using MW irradiation with 2 equiv of $NaBH_4$ at 50 °C and nicely, we found that for 10 min, excellent yield was obtained. Then, we minimize the conditions (time, temperature and equiv of $NaBH_4$) using water and water: methanol mixtures as solvents to achieve a greener method that heating conventional (Table 2, entries 4–9). In this way, the reaction under MW is optimized using only 1 equiv of $NaBH_4$ at 40 °C for 5 min in methanol (Table 2, entry 5). These excellent results (high atomic economy and short reaction time) may be being the consequence of a specific MW effects rather than a purely thermal effect that occur with a rapid elevation of temperature and pressure of system [63].

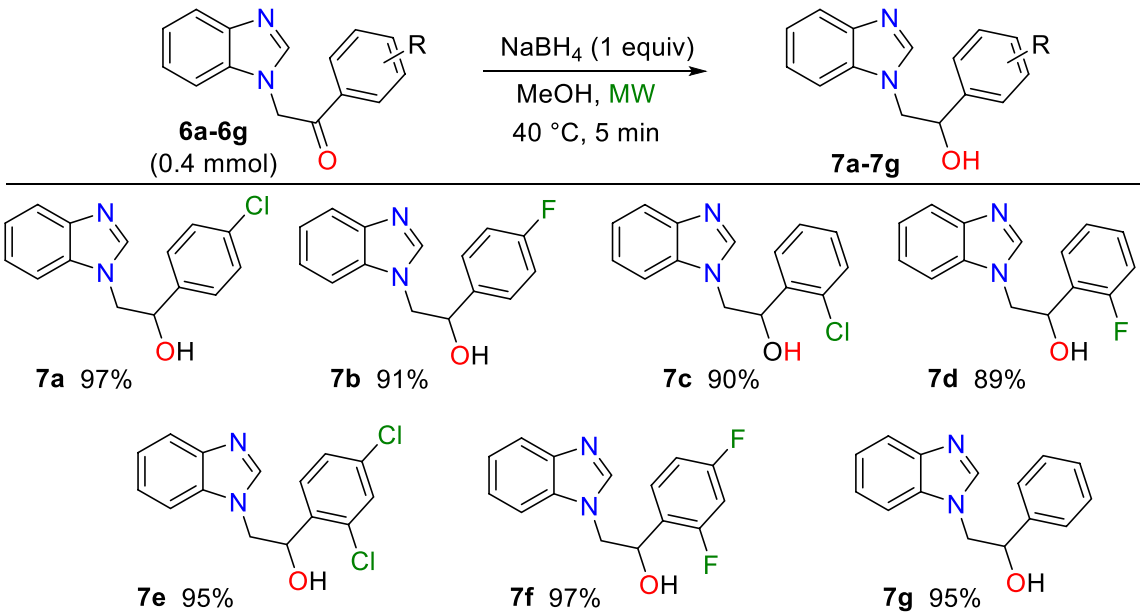
On the other hand, in the same reaction but using US irradiation at 40 °C for 10 min in 5.0 cm^3 of methanol, also an excellent yield was obtained. However, for the reaction in water, 2 equiv of $NaBH_4$ for 30 min were needed to give the expected alcohols **7a** in high yield (Table 2, entries 10–15). These reaction conditions in water have been found better to those described by conventional methodologies, where it is usually needed an excess of $NaBH_4$ in methanol with reaction times exceeding 3 h [47–50]. In this experiment, it is clear the US effect increases the solubility of reactant mixture in water, it doing a more green method, viable and efficient procedure.

With the best-established reaction conditions for the reduction of **6a** (Table 2, entry 5), we then examined the scope of this MW-assisted transformation with diverse ketones **6a**–**6g** (MB). This is the best method for its operational simplicity and greater eco-compatibility, although the method by US is also quite good. The reaction of the *N*-phenacylbenzimidazoles **6a**–**6g** (0.4×10^{-3} mol) and ~1.0 equiv of $NaBH_4$ in 1.0 cm^3 of methanol under MW gave the expected secondary alcohols **7a**–**7g** in high yields (Scheme 3). The structures of the ketones **6a**–**6g** and alcohols **7a**–**7g** were determined by HRMS analysis, ^1H spectroscopy, and ^{13}C NMR spectroscopy (see "Experimental" section).

In vitro antifungal activity

Benzimidazoles **6a**–**6g** and **7a**–**7g** were tested for antifungal properties against *C. albicans* (*Ca*) and *C. neoformans* (*Cn*) (Schemes 1, 2, 3). The antifungal activity was assessed

Scheme 3



with the broth microdilution method M27-4th edn for yeasts of the Clinical and Laboratory Standards Institute (CLSI) [68]. The percentage of inhibition of each fungus strain was determined for all compounds at the concentration range $250–3.9 \times 10^{-6}/cm^3$, which allowed the determination of MIC_{50} of each compound that represents the minimum concentration that inhibited 50% of fungal growth. Compounds with $MIC_{50} > 250 \times 10^{-6}/cm^3$ were considered inactive; with $250 \geq MIC50 > 125 \times 10^{-6}/cm^3$, marginally active; with $125 \geq MIC50 > 31.2 \times 10^{-6}/cm^3$ moderately active, and with $MIC50 \leq 31.2 \times 10^{-6}/cm^3$ highly active. Amphotericin B was used as a positive control and displayed 100% of inhibition at all concentrations tested.

Table 3 shows the MIC_{50} values of the compounds against *C. albicans* and *C. neoformans*, in which, the difference in sensitivity of each fungus to the tested compounds and some structure–activity relationships can be drawn:

1. To determine which fungus was the most sensitive to all tested compounds, the percentage of occurrence of each MIC_{50} respective to the total MIC_{50} values was recorded in a comparative graph (Fig. 2). The Fig. 2 shows that *C. neoformans* is more sensitive to all compounds, since about 86% of the 14 MIC_{50} values (column 4) were $\leq 125 \times 10^{-6}$ g/cm^3 [4 MIC_{50} of 125×10^{-6} g/cm^3 (4/14 = 28%); 7 MIC_{50} of 62.5×10^{-6} g/cm^3 (7/14 = 51%); and 1 MIC_{50} of 31.2×10^{-6} g/cm^3 (1/14 = 7%)]. Instead, only 35% of MIC_{50} values against *C. albicans* (column 3) were $\leq 125 \times 10^{-6}$ g/cm^3 (14% of $MIC_{50} = 125 \times 10^{-6}$ g/cm^3; 14% of $MIC_{50} = 62.5 \times 10^{-6}$ g/cm^3 and 7% of $MIC_{50} = 31.2 \times 10^{-6}$ g/cm^3).
2. To draw some structure–activity relationships, the percentages of inhibition of ketones **6a**–**6g** and alcohols **7a**–**7g** (Table 4) against *C. neoformans* were compared as dose–response curves in two graphs showing inhibition% (*y* axis) versus concentrations (*x* axis) (Fig. 3a, b). In Fig. 3, it is observed that alcohols display higher inhibition percentages of *C. neoformans* than ketones (left upper quadrant).
3. To analyze the role played by the different substituents in the anti-cryptococcal activity of alcohols **7a**–**7g**, they were analyzed by comparison of dose–responses curves (Fig. 4).

Figure 4 shows that the substitution of **7g** with a Cl or a F in the *p* or *o* positions (Fig. 4a, b, respectively) render more active alcohols (**7a**, **7b**, **7c**, and **7d**) and of them, the compounds with substituents in the *o* position (**7c** and **7d**, Fig. 4b) are more active than those with the same substituents in the *p* position (**7a** and **7b**, Fig. 4a). In turn, **7d** with an *o*-F substituent was the most active of the four compared compounds. However, when the phenyl ring has two halogen atoms (Fig. 4c), although the curve of both compounds **7e** (2,4-Cl_2) and **7e** (2,4-F_2) almost superimpose from 125×10^{-6} g/cm^3 to higher concentrations, compound **7e** showed the best activity since it reaches 80% of inhibition at 31.2×10^{-6} g/cm^3, while **7f** reaches only 37% inhibition at the same concentration. In summary, of the whole series of alcohols, the most active compound was **7e**, with two Cl atoms in the phenyl ring.

Conclusion

To sum up, we have established an eco-friendly and expeditious procedure to achieve a family of *N*-phenacylimidazoles **6a**–**6g** and *N*-(2-haloaryl-2-hydroxiethyl)benzimidazoles **7a**–**7g** in high yields and under mild conditions. In this synthetic approach, a comparison between US, MW, and conventional heating methods was carried out. Exceptional results were found in US for the N-phenacylation that let forming the ketones **6a**–**6g** using catalytic amount

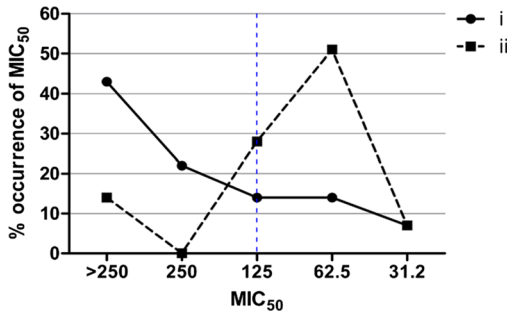
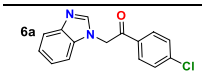
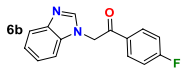
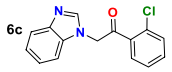
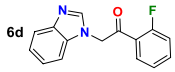
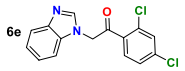
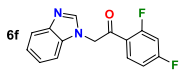
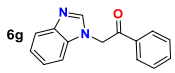
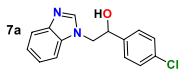
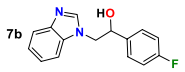
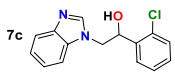
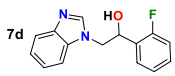
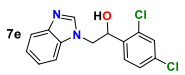
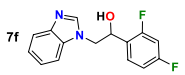
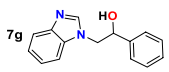


Fig. 2 Occurrence (%) of each MIC_{50} value/total MIC_{50} values of **6a**–**6g** and **7a**–**7g** against *C. albicans* (i) and *C. neoformans* (ii)[a]

[a] At right of the dotted vertical line, it can be clearly observed that for (ii) the% occurrence of MIC_{50} values $\leq 125 \times 10^{-6}$ g/cm^3, is 86% (28, 51 and 7% for 125, 62.5 and 31.2×10^{-6} g/cm^3, respectively) that against *C. albicans* that is 35% (14, 14 and 7% for 125, 62.5 and 31.2×10^{-6} g/cm^3, respectively).

Table 4 Percentages of inhibition of *C. neoformans* by compounds **6a**–**6g** and **7a**–**7g**

Compound	250	125	62.5	31.2	15.6	7.8	3.9	MIC_{50}
6a 	84.5 ± 1.2	61.8 ± 0.3	52.4 ± 1.2	36.1 ± 1.4	14.1 ± 1.3	8.7 ± 0.2	8.0 ± 0.1	62.5
6b 	79.4 ± 1.0	49.6 ± 0.3	32.8 ± 1.7	12.6 ± 0.1	12.4 ± 0.2	8.2 ± 1.1	7.6 ± 0.2	125
6c 	28.6 ± 0.4	17.7 ± 1.9	16.7 ± 0.1	16.7 ± 0.6	16.6 ± 1.1	16.3 ± 0.1	9.3 ± 0.12	>250
6d 	100	78.1 ± 0.4	60.4 ± 0.3	48.2 ± 0.5	34.0 ± 0.9	27.8 ± 0.3	5.7 ± 0.9	62.5
6e 	100	77.0 ± 1.1	51.7 ± 1.3	35.0 ± 0.3	26.0 ± 1.9	17.9 ± 0.0	4.8 ± 0.7	62.5
6f 	93.3 ± 0.4	72.5 ± 0.3	53.1 ± 0.3	39.5 ± 0.3	39.5 ± 1.7	29.0 ± 0.3	21.9 ± 1.5	62.5
6g 	74.3 ± 0.6	64.6 ± 0.4	40.7 ± 1.1	26.2 ± 0.1	25.0 ± 0.8	19.8 ± 0.1	13.5 ± 0.6	125
7a 	59.2 ± 1.5	58.9 ± 1.2	53.5 ± 0.2	47.2 ± 0.2	20.3 ± 0.5	22.9 ± 0.8	9.3 ± 0.6	62.5
7b 	77.2 ± 0.4	68.2 ± 0.5	35.8 ± 0.9	32.1 ± 0.5	18.3 ± 0.4	0.00	0.00	125
7c 	82.9 ± 0.3	61.3 ± 0.3	30.9 ± 0.3	28.3 ± 0.2	18.9 ± 1.3	18.6 ± 0.3	6.1 ± 0.7	125
7d 	89.5 ± 1.9	87.2 ± 1.1	49.1 ± 0.2	43.3 ± 0.4	33.9 ± 0.1	19.7 ± 0.2	0.00	62.5
7e 	85.2 ± 0.2	84.4 ± 0.5	83.7 ± 0.4	80.2 ± 0.9	39.7 ± 0.4	11.3 ± 0.4	0.00	31.2
7f 	94.3 ± 0.0	87.9 ± 1.1	61.4 ± 1.5	37.5 ± 0.1	32.9 ± 0.2	12.4 ± 0.0	5.86 ± 0.0	62.5
7g 	35.0 ± 0.2	34.6 ± 0.3	28.5 ± 0.4	23.1 ± 0.6	22.1 ± 0.6	20.3 ± 0.5	0.2 ± 1.2	>250
Anfotericin B	100	100	100	100	100	100	100	1.0

Cn ATCC 32264. Two-fold dilutions at the range 250–3.9 × 10^{-6} g/cm^3. MIC_{50} (in × 10^{-6} g/cm^3) value represents the minimum concentration of each compound that inhibited 50% of fungal growth

of Et_3N. The later reduction of **6a**–**6g** to obtain the final alcohols **7a**–**7g**, proceeded efficiently under MW. Both the US and MW method offer advantages with respect to other process to obtain benzimidazoles with promissory antifungal activity (atomic economy, mild reaction conditions, short reaction times, clean reactions, and high yields). Compounds **6a**–**6g** and **7a**–**7g** were tested against standardized strains of the clinically important fungi *C. albicans* and *C. neoformans* and several of them displayed moderate–good in vitro activity against *C. neoformans*, being the alcohols **7a** and **7e** showed the lowest MIC_{50} values (31.2 × 10^{-6} g/cm^3), possibly due to its greater resemblance to the recognized antifungal azoles. Apparently, the *N*-substituted benzimidazoles are a good design pattern of new analogues with improved activity; thus, we expect to extend this synthetic approach using other NH-azoles.

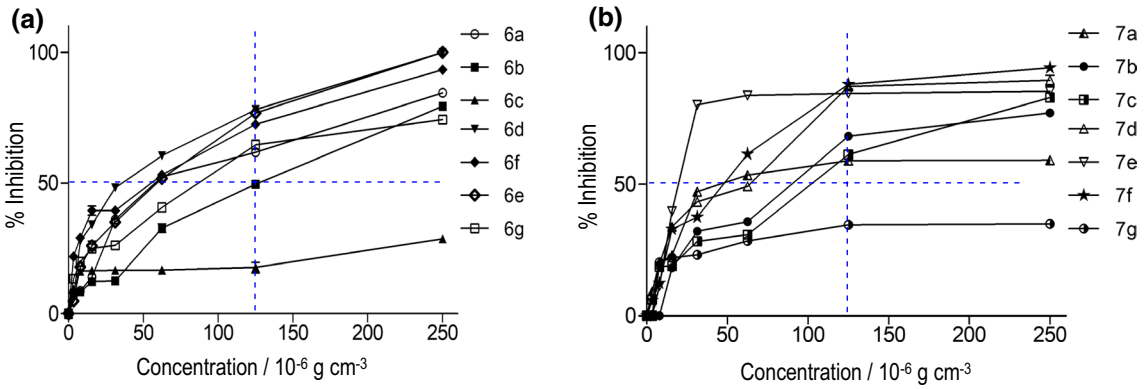
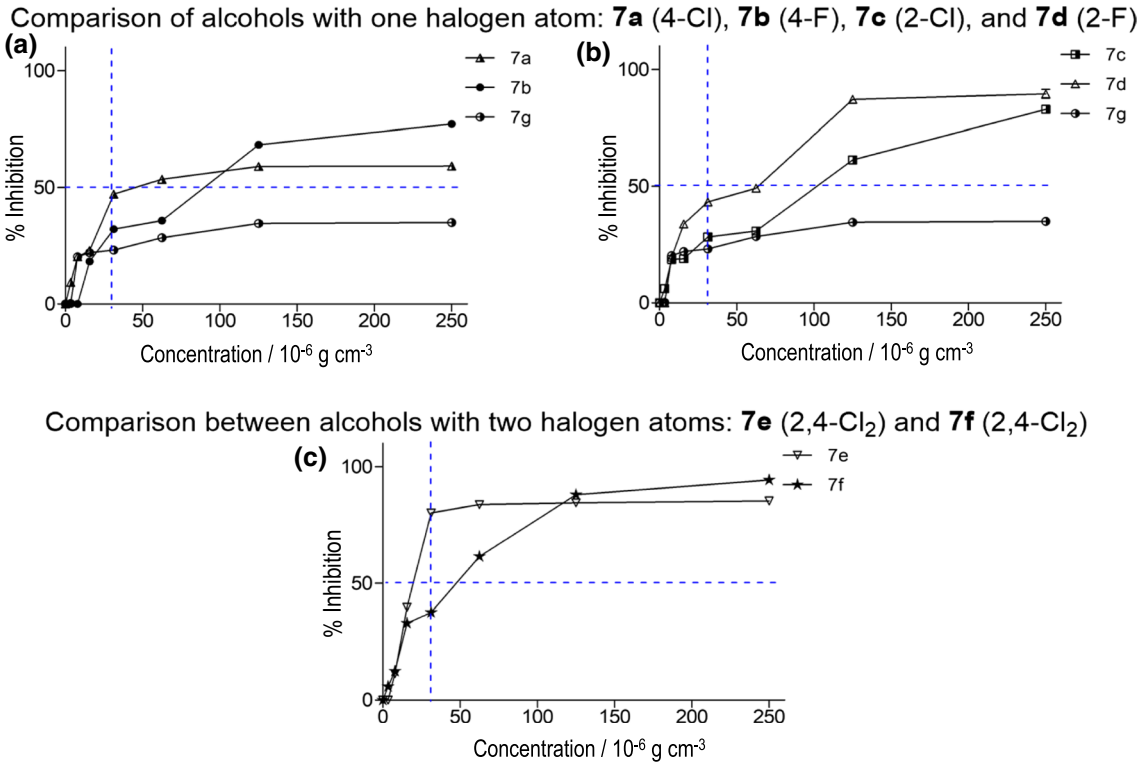


Fig. 3 Comparative antifungal dose-responses curves of ketones **6a**–**6g** (**a**) with alcohols **7a**–**7g** (**b**) against *Cryptococcus neoformans*



[a] The horizontal dotted lines show 50% inhibition and the vertical dotted line shows the concentration 31.2×10^{-6} g/cm^3.

Fig. 4 Comparative antifungal activities of the alcohols having one (**a**, **b**) or two (**c**) halogen atoms

Experimental

All reagents were purchased from commercial sources and used without further purification. Reactions were monitored by thin-layer chromatography (TLC) and visualized by an UV lamp (254 or 365 nm). Flash chromatography was performed on silica gel (230–400 mesh). US-assisted reactions were performed using an ultrasonic Sonics Vibra-Cell™ VCX 750 probe equipped with both a tapered microtip of 1⁄4″ and a thermocouple. Reactions under US were performed using a glass two necks pear shape flask heart (TNPSFH) of 25 cm^3. MW-assisted reactions were performed in a CEM Discover™ focused MW ($\nu = 2.45$ GHz) reactor equipped with a built-in pressure measurement sensor and a vertically focused IR temperature sensor. Reactions

Table 3 MIC_{50} values (in 10^{-6} g/cm^3) of **6a**–**6g** and **7a**–**7g** against *Ca* and *Cn*

Compound	*Ca*	*Cn*	Compound	*Ca*	*Cn*
6a	> 250	62.5	**6f**	250	62.5
7a	31.2	62.5	**7f**	250	62.5
6c	> 250	> 250	**6b**	250	125
7c	> 250	125	**7b**	125	125
6e	62.5	62.5	**6g**	> 250	125
7e	62.5	31.2	**7g**	> 250	> 250
6d	125	62.5	Anfotericin B	0.25	0.25
7d	> 250	62.5			

under MW were performed using a sealed reaction vessel (10.0 cm^3) containing a Teflon-coated stir bar (obtained from CEM). The NMR spectra were recorded on a Bruker Avance 400 (400.1 MHz for 1H, 100.6 MHz for 13C) at 298 K. The NMR spectroscopic data were recorded in $CDCl_3$ or DMSO-d_6 with the residual non-deuterated signal for 1H NMR and the deuterated solvent signal for 13C NMR as internal standards. The DEPT spectra were used to assign the carbon signals. The chemical shifts (δ) are reported in ppm, and the coupling constants (J) are reported in Hz. The following abbreviations are used for multiplicities: s = singlet, d = doublet, t = triplet, m = multiplet and br = broad. Melting points were determined using a capillary melting point apparatus. The high-resolution mass spectra (HRMS) were obtained on an Agilent Technologies Q-TOF 6520 spectrometer via electrospray ionization.

The detailed synthetic route of benzimidazoles **6a**–**6g** and **7a**–**7g** starting from *o*-phenylendiamine (**8**) and substituted acetophenones **9a**–**9g** is shown in Scheme S1 (Supplementary Material). Precursors 1*H*-benzimidazole (**5**) [69] and phenacyl bromides **2a**–**2g** [70–73] were prepared using some variants of the known procedures (see SI for details). The NMR data of these precursors match with previously reported data (Figs. S1–S8, SI) [70–73]. Structures of products **6** and **7** were confirmed by NMR and HRMS analysis (Figs. S9–S36, SI). The synthesis of **6a**–**6g** and **7a**–**7g** starting from 1*H*-benzimidazole (**5**) and substituted phenacyl bromides **2a**–**2g** is summarized in Scheme 1b and is described below.

General method for synthesis of *N*-phenacylbenzimidazoles 6a–6g

Method A 1*H*-Benzimidazole (**5**, 24 mg, 0.20×10^{-3} mol), 30×10^{-3} g of Et_3N (1.5 equiv) and 93×10^{-3} g of 2-bromo-4′-chloroacetophenone (**2a**, 2 equiv) in 2.0 cm^3 acetone were placed in a two-neck flask of 25 cm^3. The mixture was stirred at reflux for 1.5 h and after the completion of the reaction, the solvent was removed and the residue was purified by flash chromatography on silica gel (DCM:MeOH 3:1 v/v) to give *N*-(4-chlorophenacyl)benzimidazole (**6a**) as a white solid in 74% yield (40×10^{-3} g).

Method B (MW) A similar mixture to that of 'Method A' in 0.4 cm^3 of acetone was irradiated with microwaves at 100 °C (110 W, monitored by an IR temperature sensor) and maintained at this temperature for 30 min in a sealed tube containing a Teflon-coated magnetic stirring bar. The resulting reaction mixture was cooled to 50 °C by airflow, the solvent was evaporated under reduced pressure and the residue was purified analogously to 'Method A' to give the expected product **6a** as a white solid in 85% yield (46×10^{-3} g).

Method C (US) 1*H*-Benzimidazole (**5**, 24×10^{-3} g, 0.20×10^{-3} mol) and 70×10^{-3} g of 2-bromo-4′-chloroacetophenone (**2a**, 1.5 equiv) in 2.0 cm^3 of acetone:Et_3N (499:1 v/v. Catalytic amount of Et_3N, ~ 17 mol%) were placed in a two-neck flask of 25 cm^3. The mixture was directly irradiated with an ultrasonic probe (20×10^3 Hz, 750 W) at 55 °C and maintained at this temperature for 20 min. After the completion of the reaction, the solvent was removed under vacuum and the residue was purified analogously to 'Methods A and B' to give the pure product **6a** as a white solid in 90% yield (49×10^{-3} g).

Ketones **6a**–**6g** were synthetized in high yields under US irradiation (Method C, the best manner) using 118×10^{-3} g of 1*H*-benzimidazole (**5**, 1.0×10^{-3} mol) and 1.5 equiv of **2a**–**2g** in 6.0 cm^3 of acetone:Et_3N (499:1 v/v).

2-(Benzimidazolyl)-1-(4-chlorophenyl)ethanone (6a) By following the Method C in the reaction with 350×10^{-3} g 4-chlorophenacyl bromide (**2a**, 1.5×10^{-3} mol), the ketone **6a** was obtained as a white solid (250×10^{-3} g, 92%). M.p.: 117–120 °C (Lit. [55] 114–116 °C). NMR data matched previously reported data [55].

2-(Benzimidazolyl)-1-(4-fluorophenyl)ethanone (6b, $C_{15}H_{11}FN_2O$) By following the Method C in the reaction with 326×10^{-3} g 4-fluorophenacyl bromide (**2b**, 1.5×10^{-3} mol), the ketone **6b** was obtained as yellow crystals (229×10^{-3} g, 90%). M.p.: 173–175 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.56 (s, 2H), 7.21–7.32 (m, 5H), 7.83 (m, 1H), 7.92 (s, 1H), 8.07 (m, 2H) ppm; $^{13}C\{^1H\}$ NMR ($CDCl_3$, 100 MHz): δ = 50.4 (CH_2), 109.3 (CH), 116.4, 116.6 (CH, d, J = 22.7 Hz), 120.5 (CH), 122.6 (CH), 123.5 (CH), 130.8, 130.9 (CH, d, J = 9.5 Hz), 131.8 (C), 132.4 (C), 143.2 (C), 143.7 (CH), 165.3, 167.8 (CF, d, J = 256.8 Hz), 189.8 (C) ppm; HRMS: *m/z* calcd. for $C_{15}H_{12}FN_2O^+$ 255.0928 ($[M+H]^+$), found 255.0929.

2-(Benzimidazolyl)-1-(2-chlorophenyl)ethanone (6c, $C_{15}H_{11}ClN_2O$) By following the Method C in the reaction with 350×10^{-3} g 2-chlorophenacyl bromide (**2c**, 1.5×10^{-3} mol), the ketone **6c** was obtained as a yellow solid

(246×10^{-3} g, 91%). M.p.: 98–100 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta=5.55$ (s, 2H), 7.27–7.40 (m, 4H), 7.46–7.49 (m, 2H), 7.56 (d, $J=7.6$ Hz, 1H), 7.82 (m, 1H), 8.00 (s, 1H) ppm; ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz): $\delta=53.8$ (CH_2), 109.4 (CH), 120.5 (CH), 122.7 (CH), 123.5 (CH), 127.6 (CH), 129.9 (CH), 130.8 (CH), 131.3 (C), 133.3 (CH), 134.0 (C), 136.2 (C), 143.2 (C), 143.6 (CH), 194.8 (C) ppm; HRMS: *m/z* calcd. for $C_{15}H_{12}{}^{35}ClN_2O^+$ 271.0633 ($[M+H]^+$), found 271.0633.

2-(Benzimidazolyl)-1-(2-fluorophenyl)ethanone (6d, $C_{15}H_{11}FN_2O$) By following the Method C in the reaction with 326×10^{-3} g 2-fluorophenacyl bromide (**2d**, 1.5×10^{-3} mol), the ketone **6d** was obtained as yellow crystals (237×10^{-3} g, 93%). M.p.: 122–125 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta=5.53$ (s, 2H), 7.23–7.33 (m, 2H), 7.84–7.86 (m, 5H), 7.65 (m, 1H), 7.85 (s, 1H), 7.97 (m, 2H) ppm; ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz): $\delta=50.4$ (CH_2), 109.3 (CH), 116.7, 117.0 (CH, d, $J=22.01$ Hz), 120.5 (CH), 122.4 (CH), 122.5, 122.7 (C, d, $J=14.0$ Hz) 123.3 (CH), 125.2, 125.3 (C, d, $J=2.9$ Hz), 131.1, 131.2 (CH, d, $J=9.5$ Hz), 136.2, 136.3 (CH, d, $J=9.4$ Hz), 143.5 (C), 144.1 (CH), 161.2, 163.7 (C-F, d, $J=257.5$ Hz), 189.9, 190.4 (C, d, $J=5.2$ Hz) ppm; HRMS: *m/z* calcd. for $C_{15}H_{12}FN_2O^+$ 255.0928 ($[M+H]^+$), found 255.0932.

2-(Benzimidazolyl)-1-(2,4-dichlorophenyl)ethanone (6e) By following the Method C in the reaction with 402×10^{-3} g 2′,4′-dichlorophenacyl bromide (**2e**, 1.5×10^{-3} mol), the ketone **6e** was obtained as a yellow solid (287×10^{-3} g, 94%). M.p.: 134–137 °C (Lit. [11] 130–132 °C). NMR data matched previously reported data [11].

2-(Benzimidazolyl)-1-(2,4-difluophenyl)ethanone (6f, $C_{15}H_{10}F_2N_2O$) By following the Method C in the reaction with 353×10^{-3} g 2,4-difluorophenacyl bromide (**2f**, 1.5×10^{-3} mol), the ketone **6f** was obtained as a yellow solid (245×10^{-3} g, 90%). M.p.: 182–184 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta=5.47$ (d, $J=3.1$ Hz, 2H), 6.96–7.05 (m, 2H), 7.20–7.29 (m, 3H), 7.83 (m, 1H), 7.92 (s, 1H), 8.01 (m, 1H) ppm; ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz): $\delta=54.0$, 54.2 (CH_2, d, $J=12.5$ Hz), 105.0 (CH, t, $J=25.7$ Hz), 109.2 (CH), 113.2 (CH, dd, $J=2.9$, 21.5 Hz), 119.4 (C, dd, $J=3.7$, 14.7 Hz), 120.5 (CH), 122.5 (CH), 123.4 (CH), 133.4 (CH, dd, $J=4.4$, 11.0 Hz), 134.2 (C), 143.4 (C), 143.7 (CH), 162.0, 164.5 (CF, dd, $J=12.8$, 256.4 Hz), 166.0, 168.1 (CF, dd, $J=12.8$, 260.0 Hz), 188.6, 188.7 (C, d, $J=5.9$ Hz) ppm; HRMS: *m/z* calcd. for $C_{15}H_{11}F_2N_2O^+$ 273.0834 ($[M+H]^+$), found 273.0837.

2-(Benzimidazolyl)-1-phenylethanone (6g) By following the Method C in the reaction with 298×10^{-3} g phenacyl bromide (**2g**, 1.5×10^{-3} mol), the ketone **6g** was obtained as a white solid (224×10^{-3} g, 95%). M.p.: 149–151 °C (Lit. [56] 150–151 °C). NMR data matched previously reported data [56].

General method for synthesis of *N*-(2-hydroxyethyl) benzimidazoles 7a–7g

Method A Solid $NaBH_4$ (61×10^{-3} g, 1.60×10^{-3} mol) was added portionwise with stirring over a period of 5 min to a solution of 108×10^{-3} g *N*-(4-chlorophenacyl)benzimidazole (**6a**, 0.40×10^{-3} mol) in 5.0 cm^3 methanol to 0 °C. The stirring was continued at 50 °C for 4 h. After the reaction was complete (monitored by TLC), the volume of the reaction mixture was reduced to ~ 1.0 cm^3 under reduced pressure, and 5.0 cm^3 of water was added. The aqueous solution was extracted with ethyl acetate (3×5.0 cm^3), and the combined organic extract was dried over anhydrous $MgSO_4$. After the solvent was removed, the residue was purified by flash chromatography on silica gel (DCM:MeOH 3:1 v/v) to give *N*-[2-(4-chlorophenyl)-2-hydroxyethyl]benzimidazole (**7a**) in 80% yield (87×10^{-3} g).

Method B (MW) An equimolar mixture (0.40×10^{-3} mol) of the ketone **6a** (108×10^{-3} g) and $NaBH_4$ (16×10^{-3} g) in 1.0 cm^3 methanol was irradiated with microwaves at 40 °C (50 W, monitored by an IR temperature sensor) and maintained at this temperature for 5 min in a sealed tube containing a Teflon-coated magnetic stirring bar. The resulting reaction mixture was cooled to 25 °C by airflow and 5.0 cm^3 of water was added. The aqueous solution was extracted with ethyl acetate (3×5.0 cm^3), the organic extract was dried over anhydrous $MgSO_4$, and the solvent was removed to give **7a** in 97% yield (106×10^{-3} g).

Method C (US) A similar mixture to that of 'Method B' but in 5.0 cm^3 of methanol was directly irradiated with an ultrasonic probe (20 kHz, 750 W) at 40 °C and maintained at this temperature for 10 min. After the reaction was complete (monitored by TLC), the volume of the reaction mixture was reduced to ~ 1.0 cm^3 under reduced pressure, and 5.0 cm^3 of water was added. The aqueous solution was extracted with ethyl acetate (3×5.0 cm^3), the organic extract was dried over anhydrous $MgSO_4$, and the solvent was removed to give **7a** in high yield (102×10^{-3} g, 94%).

Alcohols **7a**–**7g** were synthetized in high yields by MW irradiation using 0.4×10^{-3} mol of **6a**–**6g**. This is the best method for its operational simplicity and greater eco-compatibility, though the Method C is also quite good.

2-(1-Benzimidazolyl)-1-(4-chlorophenyl)ethanol (7a, $C_{15}H_{13}ClN_2O$) By following the Method B in the reaction with 108×10^{-3} g *N*-(4-chlorophenacyl)benzimidazole (**6a**, 0.4×10^{-3} mol), the alcohol **7a** was obtained as a white solid (106×10^{-3} g, 97%). M.p.: 187–190 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta=4.15$–4.30 (m, 2H), 5.06 (m, 1H), 7.10 (t,

$J = 7.5$ Hz, 1H), 7.20 (t, $J = 7.6$ Hz, 1H), 7.32 (d, $J = 8.1$ Hz, 1H), 7.35 (br s, 4H), 7.40 (d, $J = 7.9$ Hz, 1H), 7.63 (s, 1H) ppm; $^{13}C\{^1H\}$ NMR ($CDCl_3$, 100 MHz): $\delta = 51.8$ (CH_2), 70.6 (CH), 109.4 (CH), 119.3 (CH), 121.2 (CH), 122.1 (CH), 126.8 (CH), 127.9 (CH), 132.7 (C), 133.5 (C), 140.0 (C–Cl), 142.8 (C), 143.5 (CH) ppm; HRMS: *m/z* calcd. for $C_{15}H_{14}{}^{35}ClN_2O^+$ 273.0789 ($[M+H]^+$), found 273.0788.

2-(1-Benzimidazolyl)-1-(4-fluorophenyl)ethanol (7b, $C_{15}H_{13}FN_2O$) By following the Method B in the reaction with 102×10^{-3} g *N*-(4-fluorophenacyl)benzimidazole (**6b**, 0.4×10^{-3} mol), the alcohol **7b** was obtained as a white solid (93×10^{-3} g, 91%). M.p.: 153–155 °C; ^1H NMR (DMSO-d_6, 400 MHz): $\delta = 4.30$–4.42 (m, 2H), 4.97 (br m, 1H), 5.94 (d, $J = 4.4$ Hz, 1H), 7.10 (t, $J = 8.8$ Hz, 2H), 7.16–7.23 (m, 2H), 7.38 (m, 2H), 7.52 (d, $J = 7.2$ Hz, 1H), 7.60 (d, $J = 7.1$ Hz, 1H), 8.04 (s, 1H) ppm; $^{13}C\{^1H\}$ NMR (DMSO-d_6, 100 MHz): $\delta = 51.9$ (CH_2), 70.9 (CH), 111.2 (CH), 115.2, 115.4 (CH, d, $J = 21.3$ Hz), 119.5 (CH), 122.0 (CH), 122.8 (CH), 128.5 (CH, d, $J = 8.1$ Hz), 134.4 (C), 138.8 (C, d, $J = 2.9$ Hz), 143.0 (C), 145.0 (CH), 160.7, 163.2 (CF, d, $J = 242.8$ Hz) ppm; HRMS: *m/z* calcd. for $C_{15}H_{14}FN_2O^+$ 257.1085 ($[M+H]^+$), found 257.1090.

2-(1-Benzimidazolyl)-1-(2-chlorophenyl)ethanol (7c, $C_{15}H_{13}ClN_2O$) By following the Method B in the reaction with 108×10^{-3} g *N*-(2-chlorophenacyl)benzimidazole (**6c**, 0.4×10^{-3} mol), the alcohol **7c** was obtained as a white solid (98×10^{-3} g, 90%). M.p.: 187–189 °C; ^1H NMR (DMSO-d_6, 400 MHz): $\delta = 4.26$ (m, 1H), 4.44 (d, $J = 13.2$ Hz, 1H), 5.23 (br s, 1H), 5.96 (d, $J = 4.2$ Hz, 1H), 7.21 (m, 2H), 7.35 (m, 2H), 7.45 (d, $J = 7.2$ Hz, 1H), 7.53 (d, $J = 7.7$ Hz, 1H), 7.59 (d, $J = 6.9$ Hz, 1H), 7.64 (d, $J = 7.7$ Hz, 1H), 8.10 (s, 1H) ppm; $^{13}C\{^1H\}$ NMR (DMSO-d_6, 100 MHz): $\delta = 50.1$ (CH_2), 67.8 (CH), 110.2 (CH), 119.4 (CH), 121.4 (CH), 122.2 (CH), 127.4 (CH), 128.0 (CH), 129.1 (CH), 129.3 (CH), 130.9 (C), 134.0 (C), 139.6 (C), 143.3 (C), 144.8 (CH) ppm; HRMS: *m/z* calcd. for $C_{15}H_{14}{}^{35}ClN_2O^+$ 273.0789 ($[M+H]^+$), found 273.0792.

2-(1-Benzimidazolyl)-1-(2-fluorophenyl)ethanol (7d, $C_{15}H_{13}FN_2O$) By following the Method B in the reaction with 102×10^{-3} g *N*-(2-fluorophenacyl)benzimidazole (**6d**, 0.4×10^{-3} mol), the alcohol **7d** was obtained as a white solid (91×10^{-3} g, 89%). M.p.: 142–144 °C; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 4.12$–4.15 (m, 1H), 4.42 (d, $J = 14.43$ Hz, 1H), 5.45 (d, $J = 8.56$ Hz, 1H), 7.02 (t, $J = 7.52$ Hz, 1H), 7.09 (t, $J = 9.36$ Hz, 1H), 7.16–7.23 (m, 2H), 7.31 (d, $J = 6.23$ Hz, 2H), 7.41 (d, $J = 8.07$ Hz, 1H), 7.67 (t, $J = 7.46$ Hz, 1H), 7.74 (s, 1H) ppm; $^{13}C\{^1H\}$ NMR ($CDCl_3$, 100 MHz): $\delta = 52.2$ (CH_2), 66.3 (CH), 109.8 (CH), 115.2, 115.5 (CH, d, $J = 22.01$ Hz), 119.6 (CH), 122.4 (CH), 123.2 (CH), 124.8, 124.9 (CH, d, $J = 3.67$ Hz), 127.6, 127.7 (CH, d, $J = 4.40$ Hz), 128.3, 128.4 (C, d, $J = 13.20$ Hz), 129.6, 129.7 (CH, d, $J = 8.07$ Hz), 133.5 (C), 142.8 (C), 143.7 (CH), 158.5, 160.9 (C–F, d, $J = 244.30$ Hz) ppm; HRMS: *m/z* calcd. for $C_{15}H_{14}FN_2O^+$ 257.1085 ($[M+H]^+$), found 257.1091.

2-(1-Benzimidazolyl)-1-(2,4-dichlorophenyl)ethanol (7e, $C_{15}H_{12}Cl_2N_2O$) By following the Method B in the reaction with 122×10^{-3} g *N*-(2,4-dichlorophenacyl)benzimidazole (**6e**, 0.4×10^{-3} mol), the alcohol **7e** was obtained as a yellow solid (117×10^{-3} g, 95%). M.p.: 185–187 °C; ^1H NMR (DMSO-d_6, 400 MHz): $\delta = 4.28$ (m, 1H), 4.45 (d, $J = 14.3$ Hz, 1H), 5.20 (br s, 1H), 6.05 (d, $J = 3.6$ Hz, 1H), 7.20 (m, 2H), 7.44 (d, $J = 8.0$ Hz, 1H), 7.50 (d, $J = 7.7$ Hz, 1H), 7.55 (d, $J = 8.3$ Hz, 1H), 7.64 (br m, 2H), 8.08 (s, 1H) ppm; $^{13}C\{^1H\}$ NMR (DMSO-d_6, 100 MHz): $\delta = 49.8$ (CH_2), 67.6 (CH), 110.2 (CH), 119.4 (CH), 121.4 (CH), 122.2 (CH), 127.6 (CH), 128.5 (CH), 129.5 (CH), 131.8 (C), 132.9 (C), 134.0 (C), 138.8 (C), 143.2 (C), 144.8 (CH) ppm; HRMS: *m/z* calcd. for $C_{15}H_{13}{}^{35}Cl_2N_2O^+$ 307.0400 ($[M+H]^+$), found 307.0399.

2-(1-Benzimidazolyl)-1-(2,4-difluorophenyl)ethanol (7f, $C_{15}H_{12}F_2N_2O$) By following the Method B in the reaction with 109×10^{-3} g *N*-(2,4-difluorophenacyl)benzimidazole (**6f**, 0.4×10^{-3} mol), the alcohol **7f** was obtained as a white solid (106×10^{-3} g, 97%). M.p.: 169–171 °C; ^1H NMR (DMSO-d_6, 400 MHz): $\delta = 4.33$–4.45 (m, 2H), 5.16 (br s, 1H), 5.95 (d, $J = 4.4$ Hz, 1H), 7.08 (t, $J = 7.7$ Hz, 1H), 7.15–7.25 (m, 3H), 7.45–7.54 (m, 2H), 7.63 (d, $J = 7.6$ Hz, 1H), 8.07 (s, 1H) ppm; $^{13}C\{^1H\}$ NMR (DMSO-d_6, 100 MHz): $\delta = 50.4$ (CH_2), 64.9 (CH), 103.6 (CH, t, $J = 26.0$ Hz), 110.2 (CH), 111.5 (CH, dd, $J = 3.7$, 20.9 Hz), 119.4 (CH), 121.4 (CH), 122.2 (CH), 125.7 (C, dd, $J = 3.7$, 13.9 Hz), 129.2 (CH, m), 134.0 (C), 143.2 (C), 144.6 (CH), 157.8, 160.3 (CF, dd, $J = 12.1$, 246.1 Hz), 160.4, 162.9 (CF, dd, $J = 12.0$, 246.1 Hz) ppm; HRMS: *m/z* calcd. for $C_{15}H_{13}F_2N_2O^+$ 275.0990 ($[M+H]^+$), found 275.0992.

2-(1-Benzimidazolyl)-1-phenylethanol (7g) By following the Method B in the reaction with 95×10^{-3} g *N*-phenacylbenzimidazole (**6g**, 0.4×10^{-3} mol), the alcohol **7g** was obtained as a white solid 91×10^{-3} g, 95%). M.p.: 110–112 °C (Lit. [11] 104–106 °C). NMR data matched previously reported data [11].

In vitro antifungal activity

Microorganisms and media

For the antifungal evaluation, standardized strains from the American Type Culture Collection (ATCC; Rockville, MD, USA), *C. albicans* ATCC 10231 and *C. neoformans*

ATCC 32264, were used. Strains were grown on Sabouraud-chloramphenicol agar slants for 48 h at 30 °C. They were maintained on slopes of Sabouraud-dextrose agar (SDA, Oxoid) and sub-cultured every 15 days to prevent pleomorphic transformations. Inocula were obtained according to reported procedures [68] and adjusted to $1–5 \times 10^3$ colony-forming units (CFU)/cm^3.

Fungal growth inhibition percentage determination

Broth microdilution techniques were performed in 96-well microplates according to the Clinical and Laboratory Standards Institute (CLSI) Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts, Approved Standard M27, 4th edn [68]. For the assay, compound-test wells (CTWs) were prepared from stock solutions of each compound in DMSO (maximum concentration 1%) that were diluted with RPMI-1640 medium to final concentrations of $250–0.98 \times 10^{-6}$/cm^3. An inoculum suspension (100 mm^3) was added to each well (final volume in the well = 200 mm^3). A growth control well (GCW) (containing medium, inoculum, and the same amount of DMSO used in a CTW, but compound free) and a sterility control well (SCW) (sample, medium, and sterile water instead of inoculum) was included for each fungus tested. Microtiter trays were incubated in a moist, dark chamber at 30 °C for 48 h for both yeasts. Microplates were read in a VERSA Max microplate reader (Molecular Devices, Sunnyvale, CA, USA). Tests were performed in triplicate. Reduction of growth for each compound concentration was calculated as follows: % inhibition = 100 − (OD_{405} CTW − OD_{405} SCW)/(OD_{405} GCW − OD_{405} SCW). The means ± SD were used to constructing the dose–response curves representing% inhibition versus concentration of each compound. The 50% inhibitory concentration 50 (MIC_{50}), that is, the concentration of each compound that caused 50% fungal growth reduction was taken as the minimum inhibitory concentration (MIC) endpoint. Amphotericin B (Sigma-Aldrich) was used as positive control.

Acknowledgements We thank the Department of Chemistry and Vicerrectoría de Investigaciones at Universidad de los Andes de Bogotá, Colombia for financial support. We express our gratitude to the Colombian Institute for Science and Research (COLCIENCIAS) for the financial support and for the doctoral scholarship conferred to D. V.-O. (Con. 617). We also acknowledge Edwin Guevara for acquiring the high-resolution mass spectra. SZ and EB thank the Universidad Nacional de Rosario, Argentina. EB acknowledges CONICET for a postdoctoral fellowship.

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