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Synthesis and *in vitro* Antifungal Evaluation of Novel *N*-Substituted 4-Aryl-2-methylimidazoles

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An efficient and regioselective synthesis of novel 4-aryl-2-methyl-*N*-phenacylimidazoles **5** by a microwave-assisted pseudo-tricomponent reaction between acetamidine hydrochloride (**3**) and α-bromoacetophenones **2** has been developed. The reduction of the carbonyl group of the ketones **5** offered the corresponding *N*-(2-hydroxyethyl)imidazoles **6** in good yields. Novel *N*-substituted imidazoles **5** and **6** were tested for antifungal activity against two clinically important fungi *Candida albicans* and *Cryptococcus neoformans*. The results showed that all compounds displayed very low activity against *C. albicans*. In contrast, compounds **5** and **6** were active against *C. neoformans*. Among them, ketones **5** showed better activity than alcohols **6**, with IC_{50} values as low as 15.6 μg/mL for some of them. The difluorinated compound **5e** showed the best activity against *C. neoformans*, followed by the dichlorinated derivative **5b**. The difluorinated compound **5e** showed the best activity against *C. neoformans*, followed by the dichlorinated derivative **5b**.

Introduction

Intramolecular carbon-nitrogen (C–N) bond formation is a crucial research topic in synthetic organic chemistry due to the wide range of applications of the N-heterocycles in industry and medicinal chemistry.[1] Imidazoles are derivatives that have great importance since they can be found in several types of drugs, natural products, metalloenzymes and functional chemicals.[1a,2] The application of the imidazoles might be attributed to their hydrogen bond donor-acceptor and amphoteric capacities, as well as their affinity for metals.[3] For example, the imidazole ring present in azole drugs is responsible for binding to the enzyme lanosterol 14α-demethylase in the ergosterol biosynthesis pathway by coordination with the iron atom of the hemoprotein.[4] In this sense, antifungal imidazole compounds such as miconazole (MCZ), ketoconazole (KTZ), clotrimazole (CTZ), econazole (ECZ) and tioconazole (TCZ) were the first azoles developed. With the exception of CTZ, most imidazoles in clinical use possess not only the imidazole ring but also the 2-aryl-2-alcoxyethyl moiety (the bold fragment joined to one of the N atoms of the imidazole ring in Figure 1).[4,5] Interestingly, this fragment is also present in the antifungal triazole derivatives itraconazole (ITZ), voriconazole (VCZ), and the highly used fluconazole (FLZ).[6] Although new azole antifungals containing a triazole ring have been further developed, the imidazoles maintain interest mainly for topical applications under different pharmaceutical forms,[7] such as econazole foam,[7a] miconazole vaginal creams,[7b] and others. Recently, two patents comprising a series of new 2,4,5-trisubstituted imidazoles with antifungal activities have been registered, reaffirming the current interest in imidazoles for antifungal therapy.[8] The structures of some representative antifungal imidazoles, as well as that of FCZ, are shown in Figure 1.

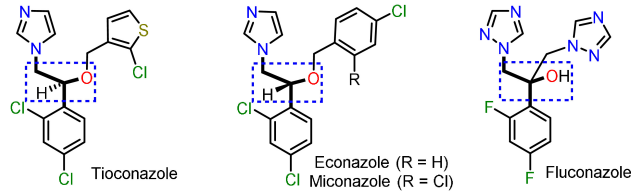

Figure 1. Azole antifungal drugs in clinical use. The 2-aryl-2-alcoxyethyl moiety present in most of them is shown in bold.

Several methods for the synthesis of novel analogues of recognized antifungal imidazoles have been developed, such as the interaction of *NH*-imidazoles with phenacyl halides (followed by a reduction reaction)[9] or 2-aryloxiranes[10] (Scheme 1a). These methods have some operational drawbacks; hence, development of alternative and efficient protocols for the preparation of novel imidazoles from inexpensive substrates is of prime importance. Currently, microwave-assisted reactions are being intensely studied due to their resemblance to the ideal synthesis criteria, since several steps can be developed in parallel and in different reaction sites leading to a unique structural assembly.[1d,11] Additionally, multicomponent reactions (MCRs) are useful synthetic tools in organic synthesis since they are efficient and proceed with high atomic economy,

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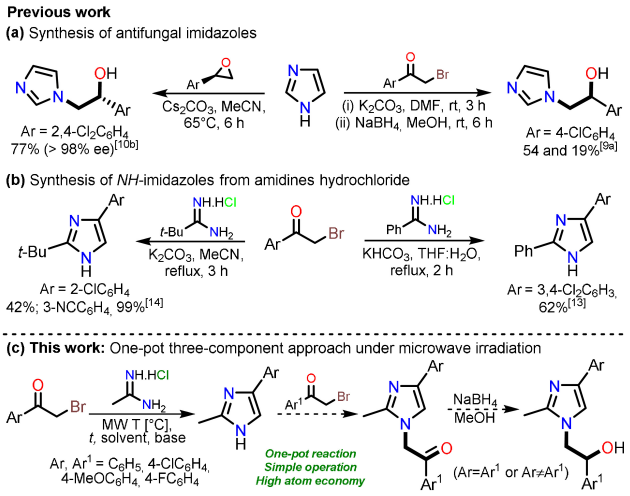


Scheme 1. Synthesis of substituted imidazoles from phenacyl bromides.

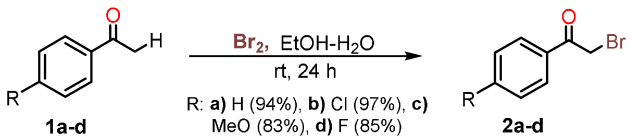
quickly and simultaneously generating molecular complexity. When two of the components are identical, the processes are described as *pseudo*-MCRs. In this case, the incorporation of two identical components into the product is a limitation in terms of scope and functional diversity. Nevertheless, these transformations have the advantage of being efficient in time, allowing on some occasions the spectacular generation of molecular complexity if the same components involve a different and complementary reactivity, which is of great interest in organic and medicinal chemistry.[12] Regarding 2,4-disubstituted imidazoles, some syntheses starting from amidine derivatives (soluble in polar organic solvents) and α-bromoacetophenones were described in previous works (Scheme 1b).[13,14] Although this protocol is reliable, many of the syntheses suffer from several drawbacks, including harsh reaction conditions, prolonged reaction times, moderate yields and limited scope; therefore, few works have reported its use. All these facts, along with our interest in developing efficient methods for intramolecular C–N bond formation,[1d,11c,12b,d,15] have led us to propose a microwave-assisted method to synthesize 2-methyl-*NH*-imidazoles starting from acetamidine (poorly soluble in organic solvents) and α-bromoacetophenones. These *NH*-imidazoles could be used as precursors of their *N*-substituted products, which have structural similarity to some azole drugs in clinical use (Scheme 1c).

Therefore, the novel products obtained in this work were tested for antifungal activity against two clinically important fungal species, *Candida albicans* and *Cryptococcus neoformans*.[16] *Candida spp.* are the fourth leading cause of nosocomial bloodstream infection (BSI) in intensive care units, causing fatal invasive candidiasis in a high percentage of patients.[16a] *C. albicans* represents more than 60% of all isolates from clinical infections. In turn, *C. neoformans* is an opportunistic fungus that causes significant mortality and morbidity mainly in HIV-infected patients, and thus, new anti-cryptococcal compounds are highly welcome.[16b]

Results and Discussion

Synthesis

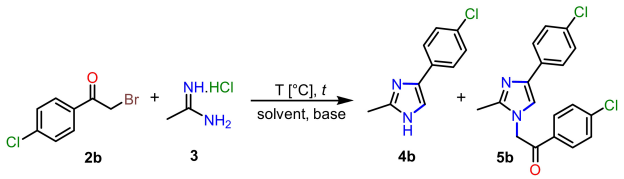
We started our work by preparing phenacyl bromides **2a–d** *via* some variations of known protocols.[17] These precursors were obtained by the bromination of different acetophenones **1a–d** in a mixture of ethanol/water (4:1 v/v) at room temperature. The products formed were filtered and dried under high vacuum to avoid its degradation at temperatures above 50 °C. The desired α-bromoketones **2a–d** were obtained with high purity and high to excellent yield after a simple purification (Scheme 2).



Scheme 2. Synthesis of α-bromoacetophenones **2a-d**. Reaction conditions: acetophenone **1** (1.0 mmol) and bromine (1.2 mmol) in EtOH/H_2O (4:1, v/v).

With the α-bromoketones precursors **2** in hand, we envisaged that the reaction between **2** and acetamidine hydrochloride (**3**) could be used to synthesize 2,4-disubstituted *NH*-imidazoles **4** (Scheme 1c). It is important to note that the amidine **3** has less steric hindrance and is less soluble in organic solvents than the corresponding benzamidine and pivalimidamide due to the effect of substituent groups (Scheme 1b). Additionally, the synthesis of *NH*-imidazoles from **3** has been very little explored. Therefore, we proposed to use microwave-assisted reactions since this method tends to favor cyclocondensation reactions using poorly soluble reagents.[11,18] In this context, we examined the reaction of **3** (0.50 mmol) with an equimolar amount of substrate **2b** to optimize this chemical transformation (see Table 1). When the reaction was heated to reflux for 24 h or was performed under microwave at 100 °C for 30 min in different polar solvents (EtOH, MeCN, H_2O or DMF), the reaction did not proceed. A similar result was seen when the reaction was performed under solvent-free conditions, using both fusion heating and microwave-assisted methods. Due to these unfavorable preliminary results, we decided to test the reaction in the presence of different bases (K_2CO_3, KOH or NaH) according to some analogous protocols.[13,14] Likewise, the solvents were chosen using the magnitude of their dielectric constants (ε_r) as a parameter, meaning we used aprotic solvents such as acetonitrile (36.7) and *N,N*-dimethylformamide (37.5) and a protic solvent such as ethanol (24.5).[14,19] Consequently, we continued by using an equimolar mixture of the reagents (**2b** and **3**) and the corresponding base (0.50 mmol). Unluckily, both at reflux (for 24 h) and under microwave irradiation (at 100 °C for 30 min) in ethanol the reaction did not proceed. However, the use of *N,N*-dimethylformamide (higher boiling temperature) as solvent under similar conditions led to the formation of a new product in poor yields (Table 1, entries 1–6). The reaction mixture was extracted and

Table 1. Optimization of the reaction parameters between 4-chlorophenacyl bromide (**2b**) and acetamidine hydrochloride (**3**).[a]



2b **3** **4b** **5b**

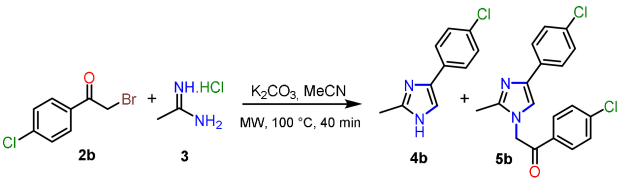
Entry	Solvent[b]	Base	T [°C]	Time, t	Yield **4b** [%]	Yield **5b** [%]
1	DMF	NaH	Reflux	24 h	–	10
2	DMF	NaH	150[d]	15 min	–	15
3	DMF	KOH	Reflux	24 h	–	10
4	DMF	KOH	150[d]	15 min	–	12
5	DMF	K_2CO_3[c]	Reflux	24 h	–	13
6	DMF	K_2CO_3[c]	150[d]	15 min	–	22
7	MeCN	NaH	Reflux	24 h	–	–
8	MeCN	NaH	100[d]	30 min	–	39
9	MeCN	KOH	Reflux	24 h	–	15
10	MeCN	KOH	100[d]	30 min	–	12
11	MeCN	K_2CO_3[c]	Reflux	24 h	–	23
12	MeCN	K_2CO_3[c]	100[d]	30 min	5	42
13	MeCN	K_2CO_3[c]	100[d]	40 min	10	46
14	MeCN	K_2CO_3[c]	120[d]	40 min	Traces	35
15	MeCN	K_2CO_3[c]	150[d]	40 min	Traces	27

[a] Conditions: 4-chlorophenacyl bromide (**2b**, 0.25 mmol), amidine **3** (0.25 mmol) and base (0.25 mmol). [b] Anhydrous solvent (0.5 mL). [c] Powdered bases. [d] Run in a 10-mL sealed tube under microwave

the residue was directly purified by flash chromatography on silica gel (eluent: DCM). The analysis by ^{1}H and ^{13}CNMR, as well as a detailed study of the HMBC spectrum of the previously purified product, indicated that the expected 2-methyl-*1H*-imidazole **4b** had not been formed. Curiously, the new isolated product corresponded to the structure of the *N*-phenacyl-2-methylimidazole **5b**, which was regioselectively obtained by a *pseudo*-tricomponent reaction. Possibly, the low solubility of acetamidine hydrochloride (**3**) decreased its reaction capacity, and the 2-methyl-*1H*-imidazole **4b** that formed would be more soluble that the precursor **3**; thus, the compound **4b** would react more easily with a second molecule of the substrate **2b**.

Consequently, we decided to carry out the reaction using acetonitrile as a solvent (similar polarity to DMF but smaller size) to avoid a poor solvation of the reagents and thus better their interaction. The reaction with sodium hydride at reflux did not proceed, but under microwave irradiation, the compound **5b** was obtained in moderate yields (Table 1, entries 7–8). When the reaction was carried out using other bases (KOH and K_2CO_3) at reflux (for 24 h) or under microwave irradiation (at 100 °C for 30 min), the *N*-phenacyl-2-methylimidazole **5b** was again obtained, but in poor to moderate yields (Table 1, entries 9–12). However, using potassium carbonate (K_2CO_3) as base under microwave irradiation at 100–150 °C for 30–40 min, it was possible to observe (by TLC) another product mixed with **5b** (Table 1, entries 12–15). The reaction mixture was extracted, and the residue was purified by flash chromatography on silica gel. The first eluted fraction (eluent: DCM) contained the new compound **5b**, while the second fraction (eluent: DCM/MeOH 20:1 v/v) contained the desired *NH*-imidazole **4b** (as minor product) whose structure was established by ^{1}H and ^{13}CNMR analysis. Thus, the best conditions for this reaction were as follows: 100 °C for 40 min under microwave along with potassium carbonate in acetonitrile (Table 1, entry 13). According to these interesting results and in order to optimize the reaction yield, we modified the above process by changing the molar equivalents of both the reagents and the base (Table 2).

Table 2. Optimization of the equivalents for the synthesis of 2-methyl-1*H*-imidazole **4b** and N-phenacyl-2-methylimidazole **5b**.[a]



2b **3** **4b** **5b**

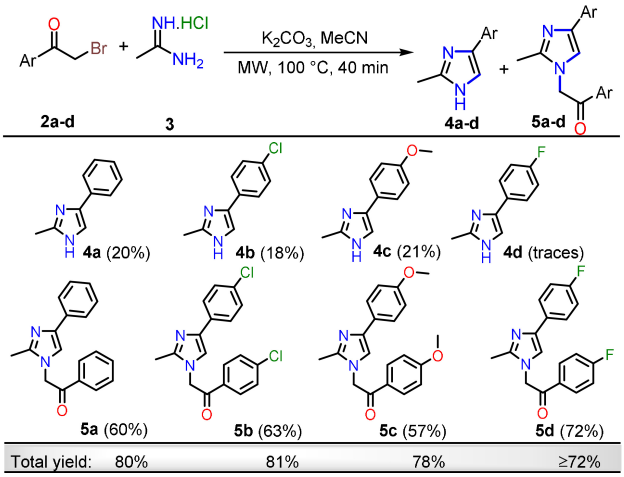
Entry	**2b**[b]	**3**[b]	K_2CO_3[b,c]	Yield **4b** [%]	Yield **5b** [%]	Yield **4b**+**5b** [%]
1	1	1	1	10	46	56
2	1	1	2	Traces	28	≥28
3	2	1	1	Traces	18	≥18
4	1	2	1	Traces	37	≥37
5	2	1	2	18	63	81
6	1	2	2	Traces	34	≥34
7	2	2	1	Traces	31	≥31
8	2	1	3	Traces	35	≥35
9	1	2	3	Traces	39	≥39
10	3	2	3	12	48	60

[a] Reactions conditions: **2b**, **3**, and K_2CO_3 (0.25 mmol) in 0.75 mL of MeCN under irradiation at the maximum power allowed by the microwave reactor in 10 mL sealed tube. [b] Equivalents. [c] Dry and pulverized K_2CO_3.

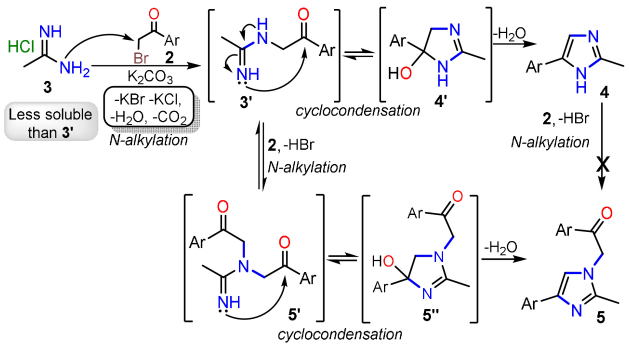
Unsurprisingly, the use of two equivalents of the substrate **2b** and of potassium carbonate, as well as of one equivalent of acetamidine hydrochloride (**3**), provided the best results (81% overall yield based on **3**, Table 2, entry 5). Reactions carried out using other ratios of equivalents afforded worse results.

In this way and with the optimized reaction conditions in hand, we then examined the scope of the reaction with different α-bromoketones **2a–d**. The microwave-assisted reaction between **3** with a set of phenacyl bromides **2a–d** afforded 2-methyl-*1H*-imidazoles **4a–d** mixed with *N*-phenacylimidazoles **5a–d** in good yields (Scheme 3). Product mixes were separated by flash chromatography on silica gel using DCM to elute the major product **5** (57–72%) and then a mixture DCM/MeOH (20:1 v/v) to elute the product **4b** (18–21%); thus, a high total yield of all reactions was obtained (72–81%). Almost no loss of efficiency was observed when the different phenacyl bromides **2a–d** were tested, which indicated the electronic demands of the substituents had little influence on the reactivity.

The formation of *N*-phenacylimidazoles **5a–d** involved a regioselective *pseudo*-tricomponent reaction following a domino sequence (N-alkylation/cyclocondensation/N-alkylation or di-N-alkylation/cyclocondensation, see Scheme 4), which allowed the formation of three new C–N bonds in one reaction step. There are few reports on the synthesis of 2-methyl-*1H*-



Scheme 3. Synthesis of *NH*-imidazoles **4 a-d** and *N*-phenacylimidazoles **5 a-d**. Conditions: α-bromoketone **2 a-d** (1.00 mmol), amidine **3** (0.50 mmol) and K_2CO_3 (1.00 mmol) in 1.5 mL of anhydrous MeCN.

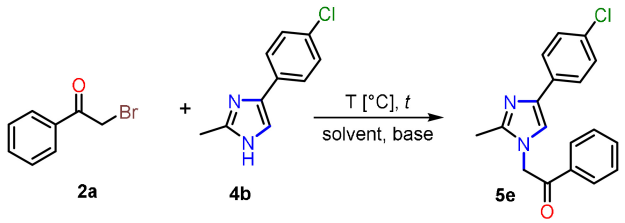


Scheme 4. Plausible mechanism for the formation of 2-methylimidazoles **4** and *N*-phenacyl-2-methylimidazoles **5**.

imidazoles **4**, and the existing methods have several operational limitations.[20] Additionally, none of these previous methods mentioned the tandem reaction (domino) discovered in this study. Likewise, to our knowledge, no reports exist about *N*-phenacyl-2-methylimidazoles **5**. These results highlight the importance of the steric effect of the amidine on the course of the reaction, since with hindered amidines (better solubility), the *NH*-imidazoles are isolated in good yields,[13,14] while with less hindered amidines (such as **3**), *N*-phenacylimidazoles **5** were isolated as the major product.

Subsequently, an *N*-alkylation of the 2-methyl-*1H*-imidazole **4 b** was carried out with phenacyl bromide (**2 a**), Table 3, due to the importance of the diversity that could be introduced in these molecules, enabling the formation of products with two different aryl groups, something that is important for studies of structure-activity relationships. According to the synthesis of products **5 a–d**, a test was carried out at room temperature and under microwave using K_2CO_3 in acetonitrile (Table 3, entries 1–2), although the product formation was not observed. However, using sodium hydride in acetone at room temperature (Table 3, entry 3) the formation of a new product was observed by TLC,

Table 3. Optimization of the synthesis of 4-aryl-*N*-phenacylimidazole **5 e**.[a]



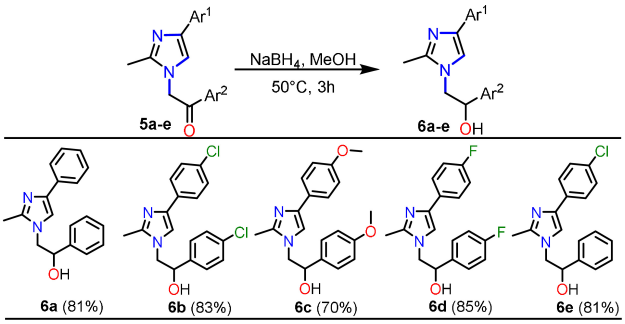
Entry	Base	Solvent	Time, t	T [°C]	Yield **5 e** [%]
1	K_2CO_3	MeCN	24 h	rt	–
2[b]	K_2CO_3	MeCN	40 min	100	–
3	NaH	Acetone	24 h	rt	10
4[b]	NaH	Acetone	15 min	50	√
5[b]	NaH	Acetone	30 min	50	√
6[b]	NaH	Acetone	15 min	100	√
7[b]	NaH	Acetone	30 min	100	√
8[b]	NaH	Acetone	40 min	100	87

[a] Reaction conditions: α-bromoacetophenone (**2 a**, 0.50 mmol), 4-aryl-2-methyl-1*H*-imidazole **4 b** (0.50 mmol) and base (0.50 mmol) in anhydrous solvent (0.5 mL). [b] Run in a 10-mL sealed tube under microwave.

the solvent was removed, and the residue was purified by flash chromatography. Then, by NMR analysis, the formation of the *N*-phenacylimidazole **5 e** was corroborated. To optimize the reaction, these steps were made under microwave irradiation with various times and temperatures (Table 3, entries 4–8), and when the absence of 2-methyl-*1H*-imidazole **4 b** was noted by TLC, isolation and purification of the *N*-phenacylimidazole **5 e** was carried out (Table 3, entry 8).

Since the *N*-alkylation did not work under similar conditions to form **5 a–d** (Table 3, entry 2), we can conclude that the effect of solubility in the course of the *pseudo*-tricomponent synthesis of compound **5** is very important. The low solubility of the smaller amidine hydrochlorides could cause the intermediate **3**′ (more soluble than **3**) to react with a second molecule of α-bromoketone **2**, forming intermediate **5**′, which would also lead to the *N*-phenacyl-2-methylimidazoles **5 a–d**; that is, there was a competition between intramolecular cyclization leading to 2-methylimidazles **4** *versus* an intermolecular reaction that would lead to the products **5**. This possibility is quite logical due to the regioselectivity observed in the reaction, as well as the nature of the reagents and the effect of solvation (Scheme 4).

Once 4-aryl-2-methyl-*N*-phenacylimidazoles **5 a–e** were obtained, and due to the importance of the 2-aryl-2-hydroxyethyl fragment in antifungal drugs, we developed a simple method to convert the carbonyl group of **5** into a hydroxyl group to generate the corresponding *N*-(2-aryl-2-hydroxyethyl)imidazole **6**. We found that the reduction reaction *via* protocols described in the literature ($NaBH_4$ in methanol)[9,11d,21] was achieved at 50 °C, giving the expected alcohols **6 a–e** in high yields (Scheme 5). The reduction on the *N*-phenacylimidazole **5 c** proceeded with the lowest yield (70%) because this substrate possessed an electron-donor group, making it exhibit less reactivity towards addition of hydrides due to the lower electrophilicity of the carbonyl carbon. In addition, by TLC we



Scheme 5. Synthesis of *N*-(2-aryl-2-hydroxyethyl)imidazoles **6a-e** *via* the reduction of the ketones **5a-e.**

noted that the reaction carried out at room temperature proceeded with low conversion.

The structures of all the compounds obtained **2a–d**, **4a–d, 5a–e**, and **6a–e** were elucidated by HRMS analysis, ^{1}H spectroscopy, and ^{13}C NMR spectroscopy. Recrystallization of the *N*-(2-hydroxyethyl)imidazoles **6a** and **6b** from a mixture of chloroform-methanol (1:1 v/v) afforded crystals of suitable size and quality for single-crystal X-ray diffraction analysis.[22] Considering the importance of this technique of structural analysis to determining the arrangement of atoms in potentially bioactive N-heterocycles, a brief description about the observed molecular conformations in the solid state of the two analyzed compounds is presented. *N*-(2-Hydroxyethyl)imidazoles **6a** and **6b** crystallized in the orthorhombic and monoclinic *Pbca* and *C*2/*c* space groups, respectively. Both molecules presented a planar conformation on the 4-arylimidazolic moiety, which was evaluated considering the dihedral angle between the mean least square planes that contained the imidazole and benzene rings. Additionally, the hydroxyethyl group (CH_2CHOH) of **6** favored intermolecular hydrogen interactions, which contributed to directing the principal fragments of the molecules to form a parallel conformation, as well as occurs in some antifungal azoles of clinical use.[5b,6b] The crystallographic data and a deeper description of the crystal structures are provided in the Supplementary Information. The Ortep drawing of these two compounds (**6a** and **6b**) is given in Figure 2, where the aforementioned molecular conformations are clarified.

Antifungal activity

In recent decades, fungi have emerged as major causes of human morbidity and mortality, mainly among immunocompromised and seriously ill hospitalized patients.[16a] Most of these mycosis-related deaths have been associated with the species *C. albicans* and *C. neoformans*. Although there are several antifungal drugs in clinical use to treat these opportunistic fungi, the fungal infections remain very difficult to eradicate. Considering that the structures of series **5** and **6** possessed a structure analogous to the imidazole antifungal drugs in clinical use,[5] eight representative compounds of both series, namely, **5a–b**, **5d–e** and **6a–d** were tested for antifungal properties against *C. albicans* and *C. neoformans*. To assess antifungal activities, the broth microdilution method, for yeasts (M27-A3 of the Clinical and Laboratory Standards Institute) was used.[23] The percentage of inhibition of each fungus was determined for each compound at two-fold dilutions in the range 250–3.9 μg/mL (Table 4). In the right column, IC_{50} represents the minimum concentration that inhibited 50% of fungal growth. This value is accepted as representative of the *in vitro* activity of target compounds.[24] Although Table 4 shows that all compounds **5** and **6** inhibited both *C. albicans* and *C. neoformans* at the different concentrations tested, it was clear that they were not good inhibitors of *C. albicans* growth (white rows) since the highest inhibition was 42.71% at 250 μg/ml (range 14.24–42.71%), with IC_{50} values $>$ 250 μg/ml for all compounds. Instead, the tested imidazoles of the series **5** and **6** were good inhibitors of *C. neoformans* growth, with all IC_{50} values in the range 15.6–250 μg/mL (percentages of inhibition ranging from 53.21 to 91.47% at 250 μg/mL). This differential behavior of all compounds against *C. albicans* and *C. neoformans* can clearly be observed in Figure 3.

A closer look at the behavior of both series against *C. neoformans* is presented in Figures 4 A and B, in which the *C. neoformans* inhibition percentages of series **5** can be compared with those of series **6**. From Figure 4, it is clear that although at 250 μg/mL both series displayed similar activities, at lower

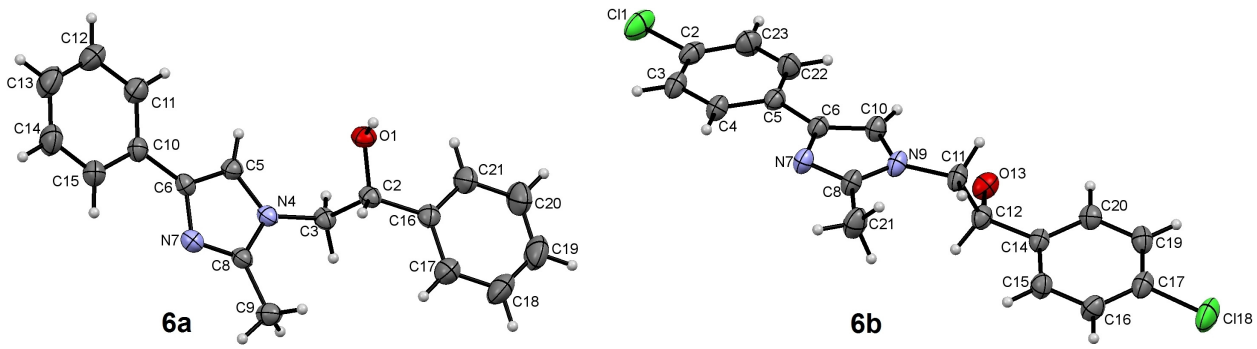
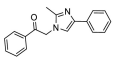
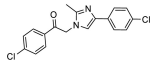
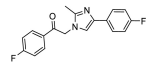
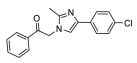
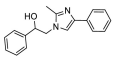
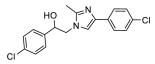
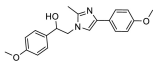
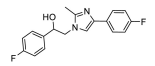


Figure 2. Ortep drawing of *N*-(2-hydroxyethyl)imidazoles **6a** and **6b**. Displacement ellipsoids are drawn at the 30% probability level and H atoms are shown as small spheres of arbitrary radius.

Table 4. Percentages of inhibition of **5a, 5b, 5d, 5e** and **6a–d** against *C. albicans* ATCC 10231 (*Ca*) and *C. neoformans* ATCC 32264 (*Cn*).[a]

	Structure	Fungi	250	125	62.5	31.2	15.6	7.8	3.9	IC_{50}
5a		Ca	32.7 ± 0.5	14.7 ± 1.5	8.6 ± 1.1	2.6 ± 0.7	2.0 ± 1.2	0.9 ± 0.1	0	>250
		Cn	71.3 ± 0.3	65.0 ± 0.2	49.6 ± 0.3	33.5 ± 0.2	31.5 ± 1.1	20.7 ± 0.2	15.8 ± 0.7	62.5
5b		Ca	41.8 ± 0.9	18.9 ± 0.1	11.0 ± 1.7	6.5 ± 1.5	2.8 ± 0.4	3.4 ± 0.0	0.2 ± 0.1	>250
		Cn	63.3 ± 0.0	61.5 ± 1.3	59.9 ± 1.2	56.2 ± 1.3	55.7 ± 0.3	28.9 ± 1.0	0	15.6
5d		Ca	42.7 ± 1.9	23.5 ± 1.1	11.3 ± 0.6	2.7 ± 0.6	1.6 ± 1.1	0.7 ± 0.4	0	>250
		Cn	91.4 ± 0.1	85.0 ± 0.2	84.3 ± 0.2	58.6 ± 0.1	49.3 ± 0.0	16.8 ± 0.1	13.5 ± 0.1	15.6
5e		Ca	24.7 ± 0.9	20.9 ± 0.7	15.5 ± 1.2	13.7 ± 0.1	11.1 ± 1.3	7.8 ± 0.7	7.3 ± 1.6	>250
		Cn	83.7 ± 0.2	78.9 ± 0.4	70.9 ± 1.4	57.0 ± 0.8	45.3 ± 0.2	39.5 ± 0.2	34.8 ± 1.5	31.2
6a		Ca	14.2 ± 0.2	5.7 ± 0.1	3.4 ± 0.6	2.2 ± 0.7	2.0 ± 0.8	2.0 ± 0.1	1.5 ± 0.1	>250
		Cn	53.2 ± 0.0	9.7 ± 0.2	0.6 ± 0.2	0	0	0	0	250
6b		Ca	26.8 ± 0.7	30.8 ± 1.0	24.7 ± 0.5	15.3 ± 0.9	10.9 ± 0.8	6.8 ± 0.9	6.1 ± 0.6	>250
		Cn	79.1 ± 0.8	76.8 ± 1.0	66.8 ± 1.7	7.5 ± 1.4	0	0	0	62.5
6c		Ca	22.5 ± 0.42	4.09 ± 0.5	0	0	0	0	0	>250
		Cn	74.5 ± 1.9	38.6 ± 0.3	0	0	0	0	0	250
6d		Ca	22.7 ± 0.7	7.5 ± 0.67	1.9 ± 0.8	1.8 ± 0.1	0	0	0	>250
		Cn	77.7 ± 1.0	70.4 ± 0.0	56.4 ± 0.8	59.3 ± 1.7	31.8 ± 0.4	13.8 ± 1.6	9.2 ± 0.3	31.2
AmpB		Ca	100	100	100	100	100	100	100	1.5
		100	100	100	100	100	100	100	1.0	

[a] Dilutions are at the range 250–3.9 μg/mL. IC_{50} value represents the concentration of each compound that inhibits 50% of fungal growth. AmpB: Amphotericin B

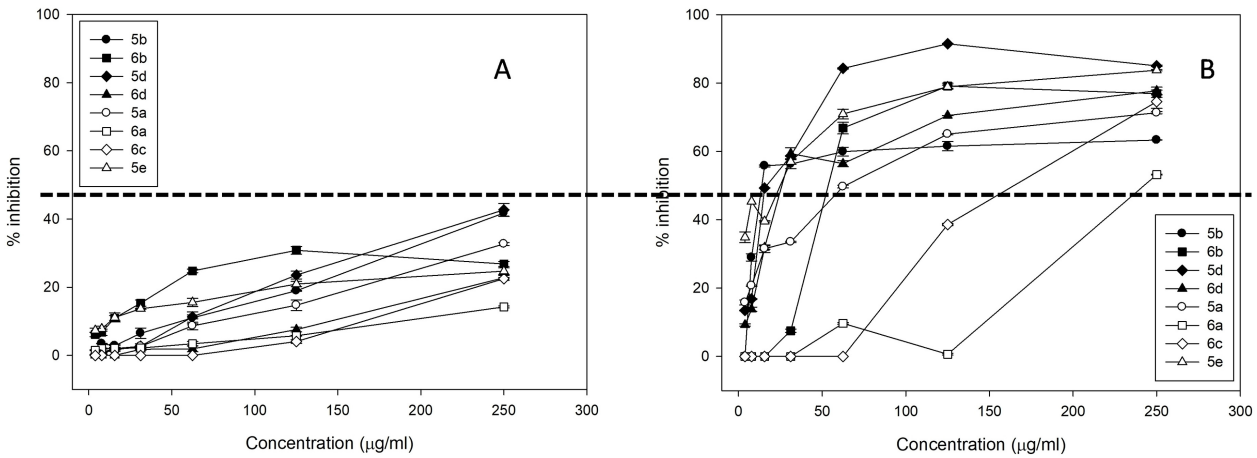


Figure 3. Comparative antifungal activities of imidazoles **5** and **6** against (A) *C. albicans* and (B) *C. neoformans*. The dotted lines were drawn for easier comparison of the compounds' effects against each fungus.

concentrations the compounds **5** were clearly more active than **6**. This can be observed at 50 μg/mL (dotted lines), where *C. neoformans* was less sensitive to compounds **6** (mainly to **6a** and **6c**) than to **5**, suggesting that the reduction of the carbonyl group to an OH group decreased the activity. Regarding the influence of the substituents of the phenyl ring on the activity of compounds **5**, the compound **5d** with two 4-F groups in its structure reached the highest inhibition percentages (Figure 4A).

Table 4 shows that **5d** displayed 91.47% inhibition of *C. neoformans* growth at 250 μg/mL, with an IC_{50} = 15.6 μg/mL. In turn, the influence of the 4-Cl substituents on the phenyl rings of compounds **5** can be observed in Figure 5A, which compares the effects of **5a** with **5d** and **5b** with **5e** against *C. neoformans*. From the curves, **5e** appears more active than **5a**, suggesting that the 4-Cl had a positive influence on the activity. In turn, structure **5b** (with two 4-Cl phenyls) was less active than **5e** at the highest tested concentrations but more active than **5e** at lower concentrations. The IC_{50} of **5b** was 15.6 μg/mL, similar to **5d** with two 4-F groups on the phenyl rings, meaning both di-halogenated compounds were the most active structures of series **5**.

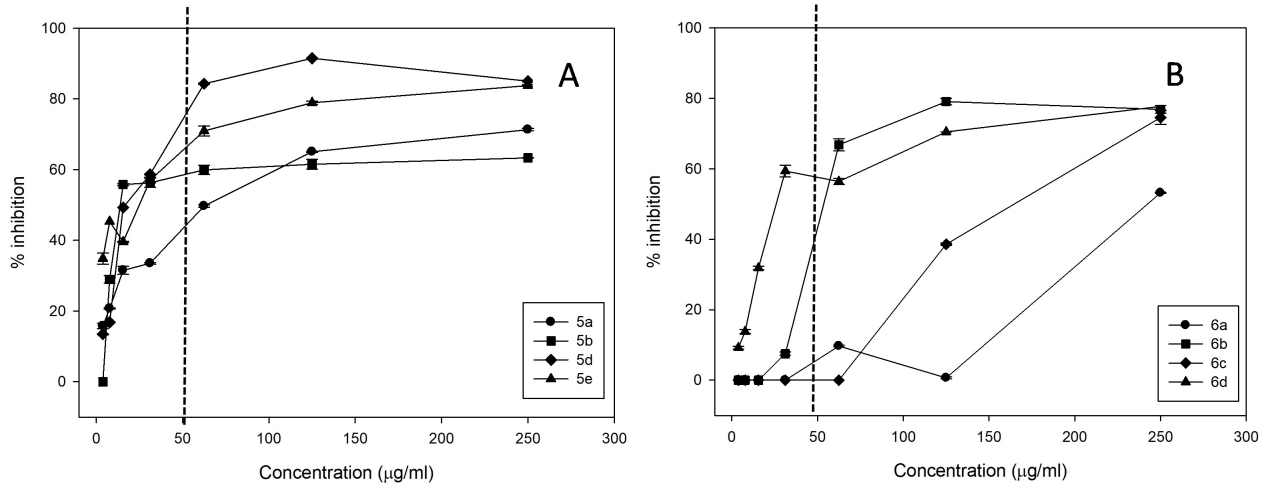


Figure 4. Comparative antifungal activities of imidazoles **5** and **6** against *C. neoformans*. The dotted lines were drawn for easier comparison of the compounds' effects at 50 μg/mL.

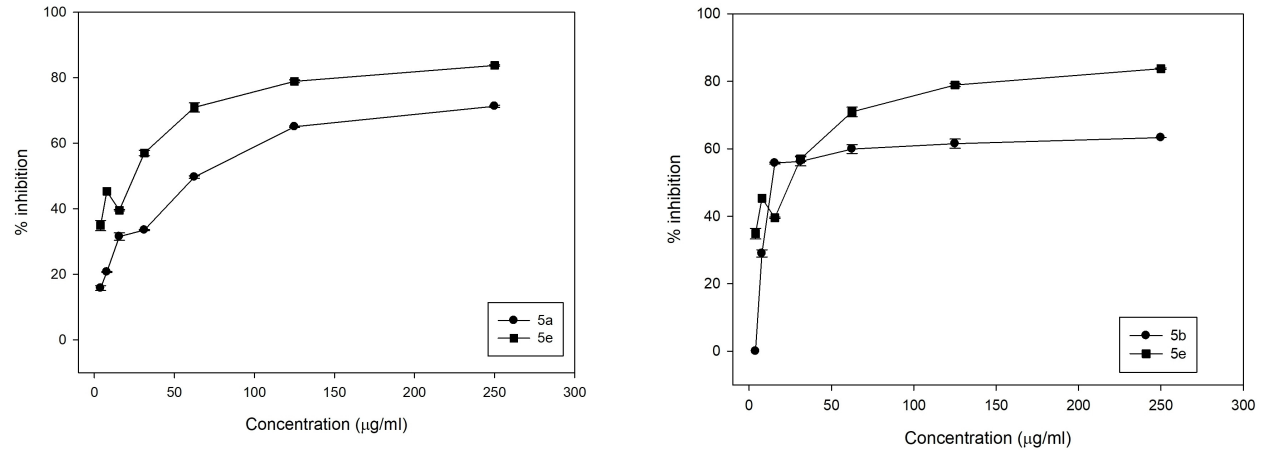


Figure 5. Comparative antifungal activities of imidazole **5a** (without any substituent on the phenyl ring) and **5b** (with a 4-Cl on each phenyl ring) with **5e** (with only one 4-Cl) against *C. neoformans*.

Conclusions

In summary, we have developed a novel and efficient microwave-assisted method for the regioselective synthesis of trisubstituted imidazoles **5** *via* the *pseudo*-tricomponent reaction of acetamidine **3** with two equivalents of α-bromoacetophenone **2a–d**. This protocol provided novel *N*-phenacylimidazoles **5a–d** in good yields (up to 72%), with the formation of three new C–N bonds in a one-pot manner, using potassium carbonate in acetonitrile. The reaction also allowed us to isolate the expected *NH*-imidazoles **4a–d** as minor products; in this way, a good total yield for the reaction was obtained (up to 81%). The *N*-phenacylimidazole **5e** with two different aryl groups was obtained through the corresponding *N*-alkylation, but this reaction only worked under microwave using sodium hydride in acetone. Therefore, the results obtained allowed us to conclude that the course of the one-pot synthesis of **5a–d** can be *via* a di-*N*-alkylation of **3** followed by a cyclocondensation reaction. In addition, the classic reduction reaction of the carbonyl group of **5a–e** was successively carried out to form the corresponding secondary alcohols **6a–e** in high yields (up to 85%). All synthesized compounds were characterized by spectroscopic analysis, and gratifyingly, the structures of the final products **6a** and **6b** were confirmed by single-crystal X-ray diffraction analysis. Trisubstituted imidazoles **5** and **6** were tested against standardized strains of the clinically important fungi *C. albicans* and *C. neoformans*. All compounds showed good activities against *C. neoformans*, though they displayed low activities against *C. albicans*. Against *C. neoformans*, ketones **5** reached better activities (IC_{50} = 15.6–62.5 μg/mL) than alcohols **6** (IC_{50} = 31.2–250 μg/mL). The dihalogenated compounds **5b** and **5e** displayed the lowest IC_{50} values (15.6 μg/mL) against *C. neoformans*, which is interesting since most antifungal azoles in clinical use have haloaryl-substituted structures. Thus, imidazoles **5b** and **5e** appear to be good models for the development of new analogues with improved

activity, and we expect to extend this method to explore reactions with other amidines and phenacyl bromides.

Supporting Information Summary

The Supporting Information contains Experimental Section, Copies of ^{1}H and $^{13}C\{^{1}H\}$ NMR spectra for all compounds, and CIFs for compounds **6 a** and **6 b**.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords: Antifungal activity · *N*-(2-Hydroxyethyl)imidazoles · Microwave synthesis · *pseudo*-Multicomponent reaction · *N*-Phenacylimidazoles

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[22] CCDC 1817753 (**6 a**) and 1817754 (**6 b**) contains the supplementary crystallographic data for this paper. Crystallographic details for these compounds are included in the Supporting Information.

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