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Synthesis of new isoxazoles and dihydroisoxazoles and in vitro evaluation of their antifungal activity

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Abstract

New 2-(2,4-dihalogenophenyl)-1-(1*H*-imidazol-1-yl)-3-(isoxazol-5-yl)propan-2-ols and 2-(2,4-dihalogenophenyl)-1-(1*H*-imidazol-1-yl)-3-(4,5-dihydroisoxazol-5-yl)propan-2-ols were synthesized by 1,3-dipolar cycloaddition between homopropargylic or homoallylic alcohols and in-situ generated nitrile oxide. Their antifungal activity was evaluated against *Candida albicans*, *C. glabrata*, *Aspergillus fumigatus* and an azole-resistant petite mutant of *C. glabrata*. Preliminary SAR results are discussed.

Keywords: *Antifungal agents, Candida albicans, Candida glabrata, conazole, isoxazole*

Introduction

Life-threatening fungal infections have been increasingly reported in recent years, especially among severely immunocompromised patients. Thus, patients undergoing neutropenic chemotherapy for organ or bone marrow transplantation, as well as patients suffering from cancer, are at high risk for invasive fungal infections [1]. Despite the discovery of echinocandins (e.g. caspofungin), a new class of antifungals that inhibit the synthesis of the major cell wall polysaccharide (1,3)-β-D-glucan [2], the current standard for treatment of systemic fungal infections still relies in the use of a polyene derivative (e.g. amphotericin B) associated or not to a nucleoside analogue (e.g. 5-fluorocytosin) or of an azole derivative (e.g. fluconazole or voriconazole) (Figure 1). Treatment with high doses of amphotericin B is however associated with significant nephrotoxocity [3], and high concentrations of 5-fluorocytosin in serum correlate with myelosuppression [4]. Therefore, azoles which present a low toxicity and a good distribution profile are usually preferred as first-line therapy [5]. As a consequence of their widespread use, it has been reported the emergence of resistant isolates. Several factors like an alteration [6] or overexpression [7] of the azole target, the cytochrome P450 dependent 14-α-demethylase, may account for the azole resistance in pathogenic yeasts. More frequently, azole resistance is associated to an increased activity of the efflux pumps leading to a suboptimal intracellular concentration of the azole drug [8]. Considering the need for new azole antifungals capable to overcome this increased efflux, we recently reported the synthesis of new 2-aryl-1-azolyl-3-thienylbutan-2-ols [9]. Some of these compounds kept a high activity against *Candida glabrata* petite mutants which are resistant to almost all the commercially available azole drugs (including fluconazole and voriconazole) because of an increased efflux, but strikingly remained susceptible to tioconazole. This study pointed out that the ability of the antifungal to overcome the increased activity of the efflux pumps is related to the presence of a five-membered heteroaromatic ring in the conazole side chain. Indeed, 4-pyridinyl and 2-quinolyl analogues of tioconazole were less active than the 2-thienyl or 3-furyl derivatives [9]. Therefore, considering the antifungal potential

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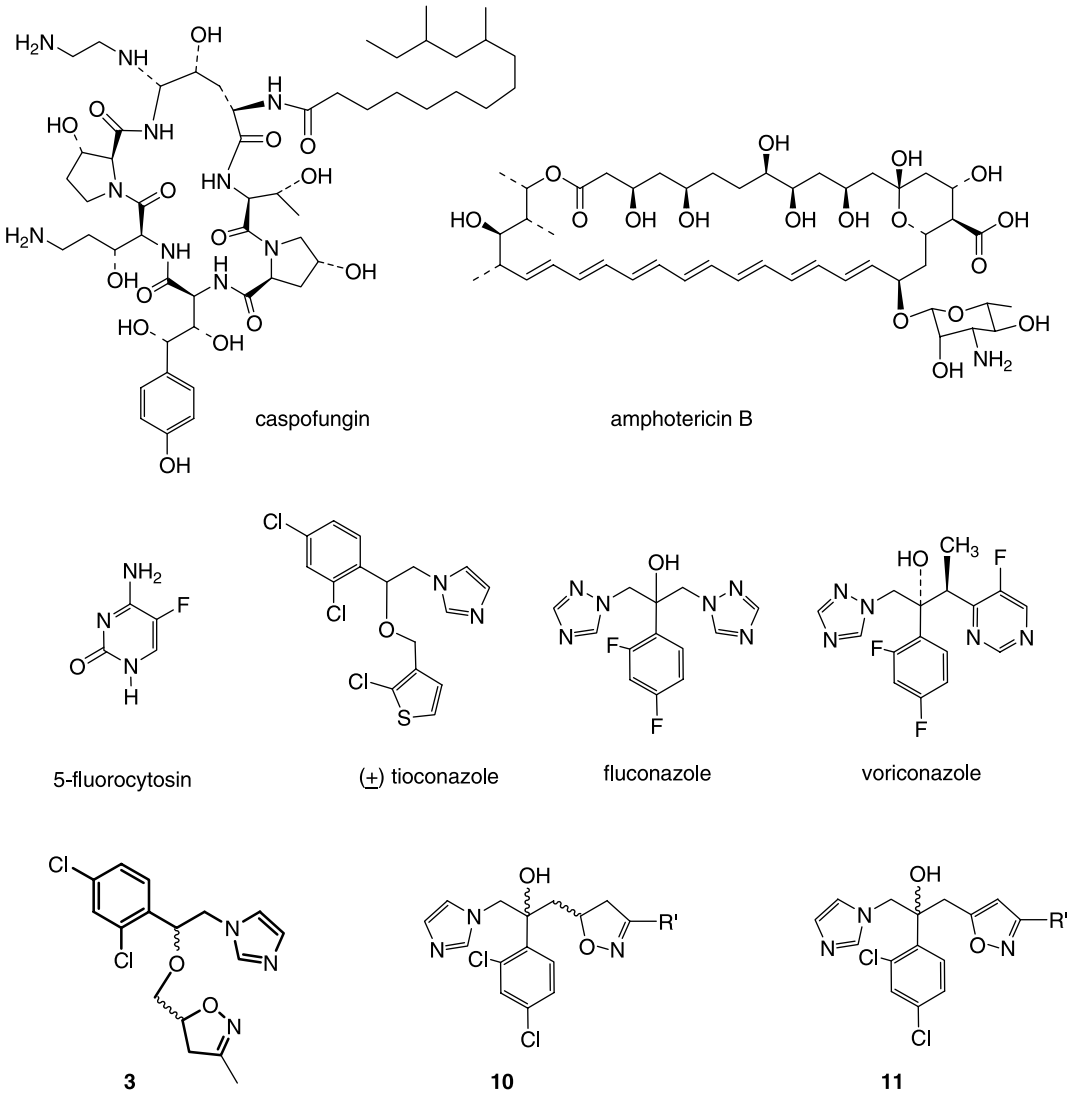


Figure 1. Antifungals used in therapy, and the general structure of the newly synthesized compounds **3**, **10** and **11**.

of oxazole derivatives [11], we would like to report herein the synthesis of new isoxazole and isoxazoline (Figure 1) in the conazole series that has rarely been studied [12,13] and an evaluation of their antifungal activity against an azole-resistant petite mutant of *Candida glabrata* and its parent wild-type strain, but also on two other human pathogenic fungi, i.e. *Candida albicans* and *Aspergillus fumigatus*.

Materials and methods

Chemistry

Instrumentation. Synthesis of compounds **2** [14], **5** [15], **6** [16] and 3,4-dimethoxybenzaldoxime [17] were achieved as previously described. Alcohol **1** [18], acetaldoxime, butyraldoxime, benzaldoxime were commercially available from Acros Organics and the ketone **4** [19] from Ugarit Chimie. Silica gel 60 (Macherey-Nagel, 230–400 mesh) was used for column chromatography and precoated Si gel plates (Macherey-Nagel, SIL G/UV254, 0.25 mm) were used for preparative TLC. Melting points were determined with an Electrothermal 8100 melting point apparatus and reported uncorrected. Infrared (IR) spectra were determined on a BRUKER FT IR Vector 22 using KBr discs for solids or neat liquid films for liquids. NMR spectra were recorded in $CDCl_3$ solution on a BRUKER AVANCE DRX 500 or a JEOL GSX 270 WB spectrometers. Chemical shifts (δ values) are expressed in parts per million downfield from tetramethylsilane as an internal standard and coupling constants (*J*) are expressed in Hertz.

*General procedure for compounds **3**, **10b**, **11b** and **11d** [20]*. To a solution of olefinic or propargylic compound **2**, **5** or **6** (1.0 mmol) and oxime **7a**–**d** (2.0 mmol) and triethylamine (2.0 mmol) in

dichloromethane (20 mL) at 0°C, was added *N*-chlorosuccinimide by small portions until complete disappearance of oxime, which was monitored by TLC. Water (20 mL) was then added to the mixture and the organic layer was washed with a 10% aqueous solution of NaOH (10 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography on SiO_2 with the adequat eluent.

*1-(2-(2,4-dichlorophenyl)-2-((4,5-dihydro-3-methylisoxazol-4-yl)methoxy)ethyl)-1H-imidazole (**3**)*. Eluent: dichloromethane/ethanol (95/5); 41%; ^{1}H-NMR (270 MHz; $CDCl_3$): 1.96 or 1.99 (s, 3H, CH_3), 2.53–2.69 (m, 1H, CH_{2a}), 2.87–2.99 (m, 1H, CH_{2b}), 3.26–3.35 (m, 1H, $CH_{2a'}$), 3.42–3.53 (m, 1H, $CH_{2b'}$), 3.96–4.07 (m, 1H, $CH_{2a''}$), 4.19–4.27 (m, 1H, $CH_{2b''}$), 4.55–4.72 (m, 1H, CH), 4.91–5.02 (m, 1H, CH), 6.96 (s, $1H_{arom}$, H-2 imidazolyl), 7.06 (s, $1H_{arom}$, H-3 imidazolyl), 7.24–7.32 (m, $2H_{arom}$, H-5′ and H-6′), 7.41 (d, $1H_{arom}$, J = 1.9 Hz, H-3′), 7.57 (s, $1H_{arom}$, H-5 imidazolyl); ^{13}C-NMR (67.5 MHz; $CDCl_3$): 13.1 (CH_3), 40.3 or 40.6 ($CH_{2/isoxazolyl}$), 51.3 (CH_2), 70.6 or 70.7 (CH_2), 77.9 or 78.1 ($CH_{isoxazolyl}$), 78.3 (CH), 127.8 (CH_{arom}), 127.9 (CH_{arom}), 128.3 (CH_{arom}), 128.5 (CH_{arom}), 129.4 (CH_{arom}), 129.5 (CH_{arom}), 132.8 ($C_{q/arom}$), 132.9 ($C_{q/arom}$), 133.4 ($C_{q/arom}$), 134.8 ($C_{q/arom}$), 154.9 and 155.0 (C = N).

*2-(2,4-Dichlorophenyl)-1-(4,5-dihydro-3-propylisoxazol-5-yl)-3-(1H-imidazol-1-yl)propan-2-ol (**10b**)*. Eluent: dichloromethane/ethanol (97/3); 23%; IR (KBr) ν_{max}: 3290 (OH), 3147 to 3058 (C–H_{arom}), 2962 to 2874 (C–H) cm^{-1}; ^{1}H-NMR (270 MHz; $CDCl_3$): 0.92 (t, 3H, J = 7.5 Hz, CH_3), 1.53 (q, 2H, J = 7.5 Hz, CH_2), 1.78 (dd, 1H, J = 11.3 and 14.5 Hz, $CH_{2a'}$), 2.26 (t, 2H, J = 7.5 Hz, CH_2), 2.49 (dd, 1H, J = 6.8 and 16.8 Hz, $CH_{2a''}$), 2.84 (dd, 1H, J = 2.5 and 14.5 Hz, $CH_{2b'}$), 2.96 (dd, 1H, J = 10.0 and 16.8 Hz, $CH_{2b''}$), 4.30 (dddd, 1H, J = 2.5-6.8-10.0 and 11.3 Hz, CH), 4.38 (d, 1H, J = 14.5 Hz, CH_{2a}), 4.45 (d, 1H, J = 14.5 Hz, CH_{2b}), 6.91 (s, $1H_{arom}$, H-2 imidazolyl), 6.93 (s, $1H_{arom}$, H-3 imidazolyl), 7.23 (dd, $1H_{arom}$, J = 2.1 and 8.7 Hz, H-5′), 7.38 (d, $1H_{arom}$, J = 2.1 Hz, H-3′), 7.57 (s, $1H_{arom}$, H-5 imidazolyl), 7.78 (d, $1H_{arom}$, J = 8.7 Hz, H-6′); ^{13}C-NMR (67.5 MHz; $CDCl_3$): 13.6 (CH_3), 19.6 (CH_2), 29.4 (CH_2), 40.1 (CH_2), 43.6 (CH_2), 54.3 (CH_2), 76.7 (C_q), 77.2 (CH), 120.3 (CH_{arom}), 127.8 (CH_{arom}), 127.9 (CH_{arom}), 130.1 ($C_{q/arom}$), 130.5 (CH_{arom}), 131.4 (CH_{arom}), 134.7 ($C_{q/arom}$), 137.5 ($C_{q/arom}$), 138.2 (CH_{arom}), 159.8 (C = N).

*2-(2,4-Dichlorophenyl)-1-(1H-imidazol-1-yl)-3-(3-propylisoxazol-5-yl)propan-2-ol (**11b**)*. Eluent: dichloromethane/ethanol (95/5); 15%; IR (KBr) ν_{max}: 3230 (OH), 3110 to 3056 (C–H_{arom}), 2962 to 2873 (C–H) cm^{-1}; ^{1}H-NMR (270 MHz; $CDCl_3$): 0.83 (t, 3H, J = 7.3 Hz, CH_3), 1.53 (s, 2H, J = 7.3 Hz, CH_2), 2.47 (t, 2H, J = 7.3 Hz, CH_2), 3.31 (d, 1H, J = 14.9 Hz, $CH_{2a'}$), 3.97 (d, 1H, J = 14.9 Hz, $CH_{2b'}$), 4.55 (d, 1H, J = 14.1 Hz, CH_{2a}), 4.84 (d, 1H, J = 14.1 Hz, CH_{2b}), 5.72 (s, $1H_{arom}$, H-4 isoxazolyl), 6.76 (s, $1H_{arom}$, H-2 imidazolyl), 6.84 (s, $1H_{arom}$, H-3 imidazolyl), 7.08 (dd, $1H_{arom}$, J = 1.9 and 8.5 Hz, H-5′), 7.37 (d, $1H_{arom}$, J = 1.9 Hz, H-3′), 7.48 (d, $1H_{arom}$, J = 8.5 Hz, H-6′), 7.73 (s, $1H_{arom}$, H-5 imidazolyl); ^{13}C-NMR (67.5 MHz; $CDCl_3$): 13.5 (CH_3), 21.4 (CH_2), 27.7 (CH_2), 34.4 (CH_2), 43.9 (CH_2), 75.8 (C_q), 103.3 ($CH_{isoxazolyl}$), 119.5 (CH_{arom}), 124.3 (CH_{arom}), 127.5 (CH_{arom}), 130.3 (CH_{arom}), 130.5 ($C_{q/arom}$), 130.7 (CH_{arom}), 133.6 ($C_{q/arom}$), 134.7 ($C_{q/arom}$), 136.5 (CH_{arom}), 155.2 ($C_{q/arom}$), 170.0 (C = N).

*2-(2,4-Dichlorophenyl)-1-(1H-imidazol-1-yl)-3-(3-(3,4-dimethoxyphenyl)isoxazol-5-yl)propan-2-ol (**11d**)*. Eluent: dichloromethane/ethanol (95/5); 12%; IR (KBr) ν_{max}: 3208 (OH), 3146 to 3018 (C–H_{arom}), 2935 to 2838 (C–H) cm^{-1}; ^{1}H-NMR (270 MHz; $CDCl_3$): 3.43 (d, 1H, J = 15.5 Hz, $CH_{2a''}$), 3.86 (s, 3H, CH_3), 3.87 (s, 3H, CH_3), 4.03 (d, 1H, J = 15.5 Hz, $CH_{2b'}$), 4.62 (d, 1H, J = 13.8 Hz, CH_{2a}), 4.89 (d, 1H, J = 13.8 Hz, CH_{2b}), 6.21 (s, $1H_{arom}$, H-4 isoxazolyl), 6.69 (s, $1H_{arom}$, H-2 imidazolyl), 6.82 (d, $1H_{arom}$, J = 8.3 Hz, H-5”), 6.87 (s, $1H_{arom}$, H-3 imidazolyl), 7.06 (dd, $1H_{arom}$, J = 1.9 and 8.7 Hz, H-5′), 7.10 (dd, $1H_{arom}$, J = 1.5 and 8.3 Hz, H-6”), 7.26 (d, $1H_{arom}$, J = 1.5 Hz, H-2”), 7.38 (d, $1H_{arom}$, J = 1.9 Hz, H-3′), 7.51 (d, $1H_{arom}$, J = 8.7 Hz, H-6′), 7.87 (s, $1H_{arom}$, H-5 imidazolyl); ^{13}C-NMR (67.5 MHz; $CDCl_3$): 38.2 (CH_2), 54.7 (CH_2), 56.2 ($2CH_3$), 72.5 (C_q), 100.4 ($CH_{isoxazolyl}$), 110.3 (CH_{arom}), 119.2 (CH_{arom}), 120.7 (CH_{arom}), 120.9 (CH_{arom}), 125.3 ($C_{q/arom}$), 128.2 (CH_{arom}), 129.1 (CH_{arom}), 130.4 (CH_{arom}), 130.6 ($C_{q/arom}$), 130.8 ($C_{q/arom}$), 132.5 (CH_{arom}), 132.9 ($C_{q/arom}$), 136.4 ($C_{q/arom}$), 149.1 ($C_{q/arom}$), 149.4 ($C_{q/arom}$), 161.5 ($C_{q/arom}$), 167.6 (C = N).

*General procedure for compounds **10a**, **10c**, **10d**, **11a** and **11c** [21]*. A aqueous solution (9.6%) of sodium hypochlorite was added dropwise to a mixture of respective olefinic or propargylic compound (1.0 mmol), respective oxime (5.0 mmol) and triethylamine (5.0 mmol) in dichloromethane (5 mL) and water (3 mL) at 20°C until complete disappearance of oxime monitored by TLC. The aqueous phase was separated and extracted with dichloromethane (20 mL). The organic phases were mixed, washed with a 10% aqueous solution of K_2CO_3 (10 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude

product was purified by flash chromatography on SiO_2 using the respective eluent.

*2-(2,4-Dichlorophenyl)-1-(4,5-dihydro-3-methylisoxazol-5-yl)-3-(1H-imidazol-1-yl)propan-2-ol (**10a**)*. Eluent: dichloromethane/ethanol (97/3); 20%; IR (KBr) ν_{max}: 3247 (OH), 3109 to 3027 (C–H_{arom}), 2982 to 2848 (C–H) cm^{-1}; ^{1}H-NMR (270 MHz; $CDCl_3$): 1.82 (dd, 1H, J = 10.7 and 14.6 Hz, $CH_{2a'}$), 1.91 (s, 3H, CH_3), 2.46 (dd, 1H, J = 7.1 and 17.1 Hz, $CH_{2a''}$), 2.79 (dd, 1H, J = 2.8 and 14.6 Hz, $CH_{2b'}$), 2.90 (dd, 1H, J = 10.2 and 17.1 Hz, $CH_{2b''}$), 4.32 (dddd, 1H, J = 2.8-7.1-10.2 and 10.7 Hz, CH), 4.37 (s, 2H, CH_2), 6.85 (s, 2H_{arom}, H-2 imidazolyl and H-3 imidazolyl), 7.20 (dd, 1H_{arom}, J = 2.1 and 8.5 Hz, H-5′), 7.35 (d, 1H_{arom}, J = 2.1 Hz, H-3′), 7.39 (s, 1H_{arom}, H-5 imidazolyl), 7.74 (d, 1H_{arom}, J = 8.5 Hz, H-6′); ^{13}C-NMR (67.5 MHz; $CDCl_3$): 12.9 (CH_3), 40.1 (CH_2), 44.9 (CH_2), 54.0 (CH_2), 76.7 (C_q), 77.1 (C*H), 120.5 (CH_{arom}), 127.7 (CH_{arom}), 127.9 (CH_{arom}), 130.1 ($C_{q/arom}$), 130.4 (CH_{arom}), 131.3 (CH_{arom}), 134.5 ($C_{q/arom}$), 137.5 ($C_{q/arom}$), 137.9 (CH_{arom}), 156.2 (C = N).

*2-(2,4-Dichlorophenyl)-1-(4,5-dihydro-3-phenylisoxazol-5-yl)-3-(1H-imidazol-1-yl)propan-2-ol (**10c**)*. Eluent: dichloromethane/ethanol (96/4); 57%; IR (KBr) ν_{max}: 3240 (OH), 3137 to 3065 (C–H_{arom}), 2986 to 2919 (C–H) cm^{-1}; ^{1}H-NMR (270 MHz; $CDCl_3$): 1.97 (dd, 1H, J = 10.6 and 14.5 Hz, $CH_{2a'}$), 2.92–3.02 (m, 2H, $CH_{2a''}$ and $CH_{2b'}$), 3.37 (dd, 1H, J = 10.2 and 16.7 Hz, $CH_{2b''}$), 4.46 (s, 2H, CH_2), 4.46–4.58 (m, 1H, CH), 6.91 (s, 1H_{arom}, H-2 imidazolyl), 7.00 (s, 1H_{arom}, H-3 imidazolyl), 7.27 (dd, 1H_{arom}, J = 1.9 and 8.7 Hz, H-5′), 7.32–7.62 (m, 6H_{arom}, H-2”, H-3”, H-4”, H-5”, H-6” and H-3′), 7.74 (s, 1H_{arom}, H-5 imidazolyl), 7.81 (d, 1H_{arom}, J = 8.7 Hz, H-6′); ^{13}C-NMR (67.5 MHz; $CDCl_3$): 40.5 (CH_2), 41.3 (CH_2), 54.4 (CH_2), 76.7 (C_q), 78.2 (CH), 120.7 (CH_{arom}), 126.6 (CH_{arom}), 128.0 (2CH_{arom}), 128.5 ($C_{q/arom}$), 128.6 (CH_{arom}), 129.6 (CH_{arom}), 130.1 ($C_{q/arom}$), 130.5 (CH_{arom}), 130.6 (CH_{arom}), 131.3 (CH_{arom}), 132.1 (CH_{arom}), 134.8 ($C_{q/arom}$), 137.3 ($C_{q/arom}$), 137.8 (CH_{arom}), 157.3 (C = N).

*2-(2,4-Dichlorophenyl)-1-(4,5-dihydro-3-(3,4-dimethoxyphenyl)isoxazol-5-yl)-3-(1H-imidazol-1-yl)propan-2-ol (**10d**)*. Eluent: dichloromethane/ethanol (97/3); 20%; IR (KBr) ν_{max}: 3302 (OH), 3109 to 3004 (C–H_{arom}), 2934 to 2838 (C–H) cm^{-1}; ^{1}H-NMR (270 MHz; $CDCl_3$): 1.92 (dd, 1H, J = 10.9 and 14.7 Hz, $CH_{2a'}$), 2.93 (m, 2H, $CH_{2a''}$ and $CH_{2b'}$), 3.35 (dd, 1H, J = 10.0 and 16.4 Hz, $CH_{2b''}$), 3.87 (s, 3H, CH_3), 3.88 (s, 3H, CH_3), 4.41 (s, 2H, CH_2), 4.49 (m, 1H, CH), 6.81 (d, 1H_{arom}, J = 8.3 Hz, H-5”), 6.88 (m, 2H_{arom}, H-2 imidazolyl and H-3 imidazolyl), 6.95 (dd, 1H_{arom}, J = 1.5 and 8.3 Hz, H-6”), 7.26 (dd, 1H_{arom}, J = 2.1 and 8.7 Hz, H-5′), 7.29 (d, 1H_{arom}, J = 1.5 Hz, H-2”), 7.39 (d, 1H_{arom}, J = 2.1 Hz, H-3′), 7.43 (s, 1H_{arom}, H-5 imidazolyl), 7.80 (d, 1H_{arom}, J = 8.7 Hz, H-6′); ^{13}C-NMR (67.5 MHz; $CDCl_3$): 40.3 (CH_2), 41.4 (CH_2), 54.1 (CH_2), 55.8 (2CH_3), 76.9 (C_q), 78.1 (CH), 108.6 (CH_{arom}), 110.4 (CH_{arom}), 120.4 (CH_{arom}), 120.6 (CH_{arom}), 121.1 ($C_{q/arom}$), 127.9 (CH_{arom}), 128.3 (CH_{arom}), 130.1 ($C_{q/arom}$), 130.5 (CH_{arom}), 131.4 (CH_{arom}), 134.7 ($C_{q/arom}$), 137.5 ($C_{q/arom}$), 138.0 (CH_{arom}), 149.0 ($C_{q/arom}$), 151.1 ($C_{q/arom}$), 157.0 (C = N).

*2-(2,4-Dichlorophenyl)-1-(1H-imidazol-1-yl)-3-(3-methylisoxazol-5-yl)propan-2-ol (**11a**)*. Eluent: dichloromethane/ethanol (95/5); 28%; IR (KBr) ν_{max}: 3210 (OH), 3131 to 3024 (C–H_{arom}), 2975 to 2846 (C–H) cm^{-1}; ^{1}H-NMR (270 MHz; $CDCl_3$): 2.12 (s, 3H, CH_3), 3.28 (d, 1H, J = 15.4 Hz, $CH_{2a'}$), 3.90 (d, 1H, J = 15.4 Hz, $CH_{2b'}$), 4.39 (d, 1H, J = 14.3 Hz, CH_{2a}), 4.75 (d, 1H, J = 14.3 Hz, CH_{2b}), 5.65 (s, 1H_{arom}, H-4 isoxazolyl), 6.71 (s, 1H_{arom}, H-2 imidazolyl), 6.74 (s, 1H_{arom}, H-3 imidazolyl), 7.07 (dd, 1H_{arom}, J = 2.1 and 8.5 Hz, H-5′), 7.35 (d, 1H_{arom}, J = 2.1 Hz, H-3′), 7.42 (s, 1H_{arom}, H-5 imidazolyl), 7.52 (d, 1H_{arom}, J = 8.5 Hz, H-6′); ^{13}C-NMR (67.5 MHz; $CDCl_3$): 11.3 (CH_3), 34.3 (CH_2), 53.9 (CH_2), 75.7 (C_q), 104.1 ($CH_{isoxazolyl}$), 120.3 (CH_{arom}), 127.4 (CH_{arom}), 127.6 (CH_{arom}), 130.3 (CH_{arom}), 130.5 ($C_{q/arom}$), 130.8 (CH_{arom}), 134.5 ($C_{q/arom}$), 136.9 ($C_{q/arom}$), 138.1 (CH_{arom}), 159.5 ($C_{q/arom}$), 167.3 (C = N).

*2-(2,4-Dichlorophenyl)-1-(1H-imidazol-1-yl)-3-(3-phenylisoxazol-5-yl)propan-2-ol (**11c**)*. Eluent: dichloromethane/ethanol (94/6); 30%; IR (KBr) ν_{max}: 3320 (OH), 3114 to 3065 (C–H_{arom}), 2991 to 2847 (C–H) cm^{-1}; ^{1}H-NMR (270 MHz; $CDCl_3$): 3.40 (d, 1H, J = 14.7 Hz, $CH_{2a'}$), 4.11 (d, 1H, J = 14.7 Hz, $CH_{2b'}$), 4.61 (d, 1H, J = 14.3 Hz, CH_{2a}), 4.86 (d, 1H, J = 14.3 Hz, CH_{2b}), 6.27 (s, 1H_{arom}, H-4 isoxazolyl), 6.74 (s, 1H_{arom}, H-2 imidazolyl), 6.88 (s, 1H_{arom}, H-3 imidazolyl), 7.10 (dd, 1H_{arom}, J = 2.3 and 8.3 Hz, H-5′), 7.40 (m, 4H_{arom}, H-3”, H-4”, H-5” and H-3′), 7.50 (d, 1H_{arom}, J = 8.3 Hz, H-6′), 7.66 (m, 2H_{arom}, H-2” and H-6”), 7.81 (s, 1H_{arom}, H-5 imidazolyl); ^{13}C-NMR (67.5 MHz; $CDCl_3$): 34.6 (CH_2), 54.2 (CH_2), 75.7 (C_q), 101.8 ($CH_{isoxazolyl}$), 126.7 (CH_{arom}), 127.7 (CH_{arom}), 128.2 (CH_{arom}), 128.8 (CH_{arom}), 129.8 (CH_{arom}), 129.9 (CH_{arom}), 130.4 ($C_{q/arom}$), 130.6 (CH_{arom}), 130.8 ($C_{q/arom}$), 132.4 (CH_{arom}), 134.9 ($C_{q/arom}$), 136.5 ($C_{q/arom}$), 162.3 ($C_{q/arom}$), 168.1 (C = N).

Antifungal activity

Tests were performed following the guidelines of the approved reference method for yeasts [22]. Antifungal activity was evaluated against an azole-susceptible strain of *C. glabrata* designated 94.5579 and its

derived azole-resistant petite mutant. MICs (Minimum Inhibitory Concentration) were determined using a microdilution assay in RPMI-1640 culture medium, inoculated with $0.-2.5 \times 10^3$ cells /mL. The test was performed using sterile 96 flat shaped-well microtitre plates. Serial two-fold drug dilutions were made in DMSO. Dilutions of the compounds were dispensed at a volume of 5 μL per well, to obtain final concentrations ranging from 250 μg/mL to the concentration where no inhibition was seen. After 48 h at 37°C, the absorbance was measured at 630 nm and $MICs_{90}$ were calculated at the minimum concentration required to inhibit at least 90% of the fungal growth compared to the drug-free control.

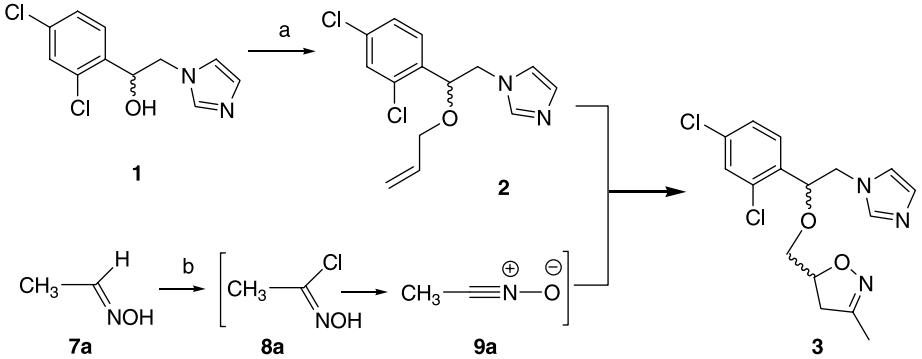
Results and discussion

Chemistry

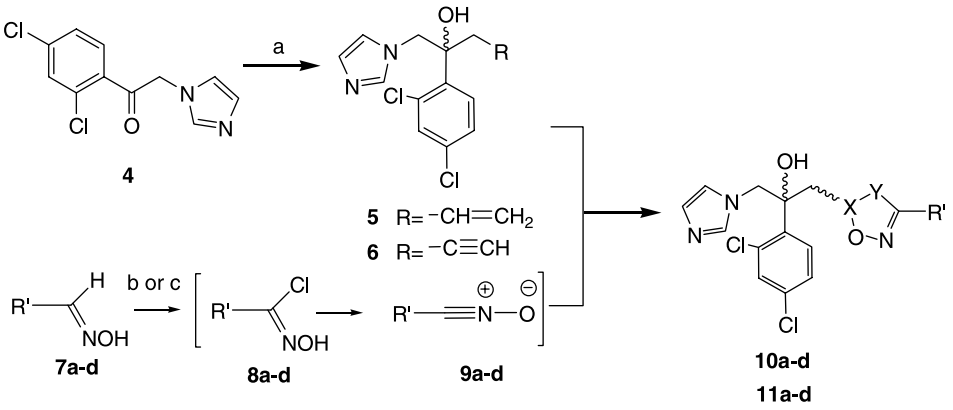
The key step in the straightforward synthesis of the oxazolines **3**, **10a**–**d** and isoxazoles **11a–d** consisted in a regioselective 1,3-dipolar cycloaddition between a nitrile oxide **9a**–**d** and the appropriate dipolarophile respectively alkenes **2**, **5** and alkyne **6**. Therefore, ether **2** was directly prepared by a classical Williamson substitution involving alcohol **1** and allyl bromide [14]. Allylic or propargylic alcohols **5** and **6** were prepared by addition of an organometallic reagent to aromatic ketone **4**. The highest yields were obtained with the less basic organozinc reagent instead with a Grignard derivative [15]. 1,3-Dipolar cycloaddition was then realized with dipolarophiles **2**, **5** and **6** and a nitrile oxide **9**. This last compound, however, is so prone to dimerize to furoxans that it is not purified usually, but generated in situ from a stable precursor such as an aldoxime **7**. Therefore, **7** was treated either by NCS [20] or NaOCl [21] to afford an hydroximinoyl chloride **8** which gives under basic condition (TEA) the corresponding nitrile oxide **9**. Its cycloaddition with the acetylenic derivative **6** led selectively to the 3,5-disusbtituted isoxazole **11** without formation of a 3,4-disusbtituted isoxazole. Dihydroisoxazoles **3** and **10** were obtained in the same way, by an 1-3 dipolar cycloaddition with **2** and **5**, respectively. Thus, an expeditious synthesis of the new isoxazolines **3**, **10** and isoxazole **11** was designed in two steps starting from the bicyclic alcohol **1** or ketone **4**, and using as a key step a selective 1,3-dipolar cycloaddition.

Antifungal activity

Taken into account the antifungal activity of tioconazole against *C. glabrata* petite mutant, compound **3**, an isoxazolinic analogue of tioconazole, was prepared. Evaluation of its antifungal activity pointed out that, contrary to several other analogues of tioconazole [10], the isoxazolinyl ether **3** was much more active against *C. glabrata* petite mutant ($MIC_{90} = 16$ μg/mL) than on its parent wild-type strain ($MIC_{90} = 125$ μg/mL). With this promising result that clearly demonstrated the sensitivity of the petite mutant to isoxazolinyl antifungal conazole, more hydrophilic compounds **10** and **11** bearing a tertiary hydroxyl group like last generation antifungal azoles (*i.e.* fluconazole or voriconazole), were synthesized. Except **11d**, all the tested azoles were active against *C. glabrata* petite mutant with MIC_{90} ranging from 2 to 62 μg/mL. However, the isoxazoles **11a–c** (MIC_{90} from 2 to 62 μg/mL) were slightly less active than the corresponding dihydroisoxazoles **10a–d** (MIC_{90} from 2 to 8 μg/mL). In the dihydroisoxazole series, aromatic (**10c**, **10d**) and aliphatic (**10a**, **10b**) 3-substituted derivatives showed the same level of activity against the mutant. In the isoxazole series, comparison of **11a** ($MIC_{90} = 8$ μg/mL) and **11b** ($MIC_{90} = 62$ μg/mL) pointed out the disfavorable effect of a long alkyl chain. However, in case of phenyl substituted isoxazoles, the substituent of the benzenic ring greatly influenced the activity. Indeed, the 3,4-dimethoxyphenyl derivative **11d** ($MIC_{90} > 250$ μg/mL) was totally inactive contrary to **11c** ($MIC_{90} = 2$ μg/mL). Moreover, in both isoxazole and dihydroisoxazole series, the phenyl substituted 5-membered ring **10c** ($MIC_{90} = 2$ μg/mL) and **11c** ($MIC_{90} = 2$ μg/mL) were the most active compounds against the azole-resistant petite mutant.



Scheme 1. Synthesis of compounds **2** and **3**. Reagents: (a) NaH, allyl bromide, THF, 90%; (b) NCS, TEA, dichloromethane, 41%.



Compound	X-Y	R'	Yield (%)
10a	CH-CH_2	Me	20
10b	CH-CH_2	Pr	23
10c	CH-CH_2	Ph	57
10d	CH-CH_2	3,4-(diOMe)C_6H_3	20
11a	C=CH	Me	28
11b	C=CH	Pr	15
11c	C=CH	Ph	30
11d	C=CH	3,4-(diOMe)C_6H_3	12

Scheme 2. Synthesis of compounds **5**, **6**, **10a**–**d** and **11a**–**d**. Reagents: (a) RCH_2ZnBr, THF, R = C_2H_3 85%, R = C_2H 53%; (b) NCS, TEA, dichloromethane; (c) NaOCl, TEA, dichloromethane.

Regarding the parent wild-type strain of *C. glabrata*, all the tertiary alcohols were more active than ether **3** (MIC_{90} = 125 μg/mL) with MIC_{90} ranging from 1 to 31 μg/mL. In addition, contrary to **3**, the hydroxyl derivatives **10** and **11** were equally efficient against the parent strain and its derived azole-resistant petite mutant. Against *C. albicans*, the phenyl substituted **10c** and **11c** were the most active tertiary alcohols with MIC_{90} value similar to fluconazole (MIC_{90} = 2 μg/mL). Moreover, except for **10b** which presented a slight activity against *A. fumigatus* (MIC_{90} = 62 μg/mL), the antifungal profile of the tested compounds **10** and **11** was similar to fluconazole. In conclusion, the antifungal activity of novel 2-aryl-1-(1*H*-imidazol-1-yl)-3-(isoxazol-5-yl)propan-2-ol against the azole-resistant petite mutant confirmed that the nature of the five membered heterocyclic ring in the side chain is an important parameter to consider in order to design new azoles capable to overcome the increased activity of the efflux pumps which is the major mechanism of the acquired resistance to azoles in clinical isolates. This study also demonstrated the antifungal potential of 2-aryl-1-azol-1-ylpropan-2-ol bearing a phenyl substituted 5-membered heterocyclic ring. Therefore, such derivatives will be now synthesized in a thiophenic series. Indeed, we recently reported that 2-aryl-1-azolyl-3-thienylbutan-2-ols could be considered as potent broad-spectrum antifungal agents also capable to overcome an increased efflux.

Table I. MIC_{90} for the tested compounds against several fungal strains.

Compound	MIC $^{\star}_{90}$ (μg/mL)			
	C. glabrata		*C. albicans*	*A. fumigatus*
	Parent	Mutant		
3	125	16	–	–
10a	8	4	16	>250
10b	4	8	16	62
10c	1	2	8	>250
10d	8	4	8	>250
11a	31	8	16	>250
11b	4	62	16	>250
11c	4	2	2	>250
11d	31	>250	31	>250
tioconazole	4	0.5	8	0.5
fluconazole	8	> 250	2	>250
voriconazole	0.5	> 250	0.062	8

⋆Minimun inhibitory concentration required to inhibit at least 90% of the fungal growth compared to the drug-free control.

Acknowledgements

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