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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and in vitro biological evaluation of 1,3-bis-(1,2,3-triazol-1-yl)-propan-2-ol derivatives as antifungal compounds fluconazole analogues

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Abstract

A novel series of 1,3-bis-(1,2,3-triazol-1-yl)-propan-2-ol derivatives was synthesized from 1-aryl-1,3-diazidopropan-2-ol derivatives and diverse alkynes using copper catalyzed azide-alkyne cycloaddition in the key step. Most of synthesized compounds showed high activity against *Candida spp*. strains at a 0.04–0.5 μg/mL concentration range compared to Itraconazole and Fluconazole (MIC 2.56 and 1.28 μg/mL, respectively), which were used as reference compounds. A 1,3-bis-(1,2,3-triazol-1-yl)-propan-2-ol derivative ($R^1 = F$ and $R^2 =$ cyclopropyl) displayed an outstanding selectivity against *Candida albicans* and *Candida krusei* (MIC = 0.0075 μg/mL). Moreover, *Artemia salina* bioassay on 1,3-bis-(1,2,3-triazol-1-yl)-propan-2-ol derivatives revealed low toxicity in this kind of compounds. In addition, molecular docking studies suggest good binding affinity of halogen atoms in some 1-aryl-1,3-diazidopropan-2-ol derivatives to HEME group present in 14-alpha demethylase (CYP51), which might explain the high antifungal activity found in these compounds.

Keywords 1,2,3-Triazoles · Antifungal · Click chemistry · Fluconazole analogues

Introduction

Triazoles are important heterocyclic rings, which integrate the structure of several pharmacologically active compounds from anticancer agents to antibiotics (Lass-Flörl 2011; Dismukes 1988; Saag and Dismukes 1988). A representative example of this kind of molecules is Fluconazole (**1**) (Richardson et al. 1985; Heeres et al. 2010), a leading drug for fungal infections treatment, which works as 14α-demethylase inhibitor at fungi cell membrane level (Sagatova et al. 2015), with favorable pharmacokinetic and pharmacodynamic characteristics that make Fluconazole an effective, powerful, safe, and low toxicity compound (Andes and van Ogtrop 1999; Rogers and Galgiani 1986). However, the emergence of new pathogens as well as resistance to Fluconazole (Dutcher 2008; Warnock et al. 1988) have promoted the development of modifications on its structure aimed to broaden the antifungal activity spectrum as well as potency increasing. Thus, several Fluconazole analogues have been designed and synthesized from the 2-aryl-1-(1,2,4-triazolyl)propan-2-ol structure presenting significant antifungal activity (Wang et al. 2014, Subhas

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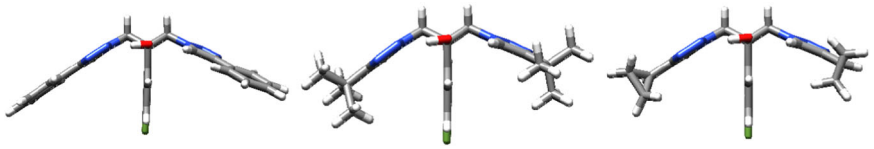


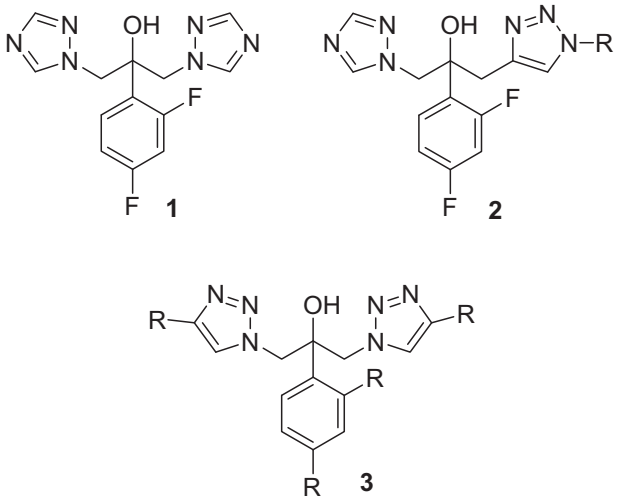
Fig. 1 Geometries of minimal energy for the proposed compounds with antifungal activity, calculated at the PBE0/3-21G level of theory: **7** left, **8** center and **9** right

et al. 2012; Zhang et al. 2011; Xu et al. 2009; Lebouvier et al. 2007; Silvestri et al. 2004; Heravia and Motamedi 2004; Eto et al. 2000; Dickinson et al. 1996).

On the other hand, copper-catalyzed azide-alkyne cycloaddition (CuAAC), the most important click reaction (Meldal and Tornoe 2008; Moses and Moorhouse 2007; Bock et al. 2006), represents a promissory source of biologically active compounds (Bonandi et al. 2017; Thirumurugan et al. 2013, Haider et al. 2014, Agalave et al. 2011), and especially, molecules with antifungal activity due to recent works, which have described this property in different 1,4-disubstituted 1,2,3-triazoles derived from this click reaction (Irfan et al. 2015; Aufort et al. 2008).

In this regard, CuAAC reaction has been successfully used for the preparation of Fluconazole derivative libraries, proving to be a powerful synthetic tool through a strategy based on a heterocyclic ring change where one of the 1,2,4-triazole rings was substituted by 1,2,3-triazole prepared from click chemistry obtaining derivatives from 2-aryl-1-(1,2,4-triazolyl)-3-(1,2,3-triazolyl)propan-2-ol (**2**), which displayed important activity against diverse human pathogenic fungi (Wang. et al. 2014; Pore et al. 2012; Zou et al. 2012, Aher et al. 2009; Pore et al. 2006).

Inspired by these facts, we propose a novel approach, which would allow the synthesis of bis(1,2,3-triazolyl)propan-2-ol derivatives (**3**) as Fluconazole analogues with both 1,2,4-triazole rings substituted by the 1,2,3-triazole moiety (Fig. 1). This report summarizes our most recent findings in the click chemistry area with the purpose to search and develop new molecules with high antifungal activity using simplest synthetic methodologies according to click chemistry philosophy. (Scheme 1)



Scheme 1 Structure of Fluconazole **1**, 2-aryl-1-(1,2,4-triazolyl)-3-(1,2,3-triazolyl)propan-2-ol derivatives **2** and general structure for molecules **3** proposed in this work

Materials and methods

General remarks

The starting materials were purchased from Aldrich Chemical Co. and were used without further purification. Solvents were distilled before use. Silica plates of 0.20-mm thickness were used for thin layer chromatography. Melting points were determined with a Krüss Optronic melting point apparatus and they are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded using a Bruker Avance 300 MHz, and a Varian 500 MHz; the chemical shifts (δ) are given in ppm relative to TMS as internal standard (0.00). For analytical purposes the mass spectra were recorded on a Shimadzu GCMS-QP2010 Plus in the EI mode, 70 eV, 200 °C via direct inlet probe. Only the molecular and parent ions (*m/z*) are reported. IR spectra were recorded on a Bruker TENSOR 27 FT instrument.

Synthesis of 1,3-diazido-propan-2-one (5)

Typical procedure. 1,3-dichloroacetone (2.0 g, 16.0 mmol) was added to a stirred mixture of NaN_3 (5.2 g, 7.98 mmol) and acetone (30 mL), and the resulting mixture was stirred at room temperature for 12–15 h. The mixture was filtered and the solvent was removed under reduced pressure. The compound was extracted with methyl tertbutyleter (2 × 30 mL), the organic layers were joined and dried over Na_2SO_4 and the solvent was removed under reduced pressure to yield 1,3-diazido-propan-2-one as a pale solid (2.1 g, 14.34 mmol, 91%), which was used without further purification. IR (ATR) ν_{max} 2104, 1757 cm^{-1}. ^{1}H NMR: (300 MHz, $CDCl_3$)

δ = 4.086 (s, 4 H). ^{13}C NMR: (75 MHz, $CDCl_3$) δ = 199.9 (1C), 57.6 (2XCH_2). MS [EI+] m/z (%): 140 $[M]^+$ (50).

General procedure of synthesis of 1,3-bis-(1,2,3-triazol-1-yl)-propan-2-ol derivatives

A solution of the appropriate arylmagnesium bromide (15.91 mmol, 2.0 M in THF) and CH2Cl2 (15 mL) was cooled to −75 °C. A solution of 1,3-diazido-propan-2-one (2.13 g, 15.91 mmol) in CH_2Cl_2 (15 mL) was added dropwise and the resulting mixture was stirred under an inert atmosphere at −75 °C for 4 h and at room temperature for 24 h. A saturated solution of NH_4Cl (40 mL) was added and the mixture was stirred for 30 min. The organic layer was separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 15 mL). The organic layers were joined and dried over Na_2SO_4 and the solvent was removed under reduced pressure to yield the corresponding 1,3-diazido-2-arylpropan-2-ols, which were used without purification. A solution of the corresponding alkyne (17.0 mmol) in MeOH (15 mL) was added to a solution of the appropriate 1,3-diazido-2-arylpropan-2-ol (11.43 mmol) in MeOH (10 mL). The mixture was stirred at room temperature for 5 min, a 2 N solution of NaOH (2 mL, 0.160 g, 4.0 mmol) and CuI (0.3063 g, 2.29 mmol) were successively added, and the resulting mixture was stirred at room temperature for 24 h. The solvent was removed under reduced pressure and concentrated NH_4OH solution (40 mL) was added and the mixture was stirred for 2 h. The product was filtered, washed with H_2O (20 mL) and cold MeOH (20 mL), and dried under reduced pressure to afford the corresponding1,3-bis-(4-cyclopropyl-1,2,3-triazol-1-yl)-2-arylpropan-2-ol derivative, which was purified by crystallization.

2-(4-Fluoro-phenyl)-1,3-bis-(4-phenyl-[1,2,3]triazol-1-yl)-propan-2-ol (7)

1,3-Diazido-2-(4-fluoro-phenyl)-propan-2-ol and phenylacetylene afforded 2-(4-Fluoro-phenyl)-1,3-bis-(4-phenyl-[1,2,3]triazol-1-yl)-propan-2-ol as a white solid. Yield: 4.52 g (90%). IR (ATR) ν_{max} 3575, 3092, 1487 cm^{-1}. ^{1}H NMR: (300 MHz, DMSO-d_6) δ = 8.31 (s, 2H), 7.82 (d, J = 6.6 Hz, 4H), 7.55 (m, 2H), 7.46 (m, 4H), 7.35 (d, J = 6.3 Hz, 2H), 7.14 (t, J = 7.5 Hz, 2H), 6.47 (s, 1H), 4.98 (d, J = 14.1 Hz, 2H), 4.81 (d, J = 14.3 Hz, 2H). ^{13}C NMR: (75 MHz, DMSO-d_6) δ = 163.5759–160.3528 (C, $J_{C\text{-}F}$ = 241.73 Hz), 146.20 (2XC), 136.86 (C), 131.12 (2XC), 129.39 (2C), 128.49–128.60 (CH, $J_{C\text{-}F}$ = 8.49 Hz), 128.30 (4XCH), 125.57 (4XCH), 123.10 (2 X CH), 115.26–114.9819 (CH, $J_{C\text{-}F}$ = 21.02 Hz), 75.15 (C), 57.61 (2 X CH_2). MS [EI+] *m/z* (%): 440$[M]^+$ (10), 363 $[M - C_6H_5]^+$ (100). HRMS (EI): for $C_{25}H_{21}FN_6O$ calcd. 440.1761, found 440.1765.

1,3-Bis-(4-cyclopropyl-[1,2,3]triazol-1-yl)-2-(4-fluorophenyl)-propan-2-ol (8)

1,3-Diazido-2-(4-fluoro-phenyl)-propan-2-ol and cyclopropylacetylene afforded 1,3-Bis-(4-cyclopropyl-[1,2,3]triazol-1-yl)-2-(4-fluoro-phenyl)-propan-2-ol as a white solid. Yield: 122.5 mg (61%). IR (ATR) ν_{max} 3539, 3074, 1483 cm^{-1}. ^{1}H NMR: (300 MHz, DMSO-d_6) δ = 7.51 (s, 2H), 7.41 (d, J = 8.3 Hz, 2H), 7.10 (d, J = 8.3 Hz, 2H), 6.23 (s, 1H), 4.74 (d, J = 14.1, 2H), 4.69 (d, J = 14.0 Hz, 2H), 1.89 (q, 2H), 0.87 (d, J = 6.6 Hz, 2H), 0.65 (s, 2H). ^{13}C NMR**:** (75 MHz, DMSO-d_6) δ = 163.49–160.26 (C, $J_{C\text{-}F}$ = 242.19 Hz), 148.67 (2XC), 136.97 (1C), 128.52–128.41 (2XCH, $J_{C\text{-}F}$ = 7.94 Hz), 122.48 (2XCH), 115.13–114.85 (2XCH, $J_{C\text{-}F}$ = 21.0675 Hz), 75.06 (C), 57.2965 (2XCH_2), 8.08 (2XCH), 6.90 (4XCH_2). MS [EI+] *m/z* (%): 368$[M]^+$ (23), 246 $[M - C_6H_8N_3]^+$ (100). HRMS (EI): for $C_{19}H_{21}FN_6O$ calcd. 368.1761, found 368.1759.

1,3-Bis-(4-tert-butyl-[1,2,3]triazol-1-yl)-2-(4-fluorophenyl)-propan-2-ol (9)

1,3-Diazido-2-(4-fluoro-phenyl)-propan-2-ol and 3,3-Dimethyl-but-1-yne afforded 1,3-Bis-(4-tert-butyl-[1,2,3]triazol-1-yl)-2-(4-fluoro-phenyl)-propan-2-ol as a white solid. Yield: 152.9 mg (63%). IR (ATR) ν_{max} 3533, 3076, 1485 cm^{-1}. ^{1}H NMR: (300 MHz, DMSO-d_6) δ = 7.48 (s, 2H), 7.38 (d, J = 8.5 Hz, 2H), 7.09 d, (J = 8.3 Hz, 2H), 6.25 (s, 1H), 4.77 (d, J = 14.1, 2H), 4.64 (d, J = 14.1 Hz, 2H), 1.22 (s, 18H). ^{13}C NMR: (75 MHz, DMSO-d_6) δ = 163.52–160.29 (C, $J_{C\text{-}F}$ = 242.07 Hz), 156.00 (2XC), 137.08 (C), 128.48–128.37 (2XCH, $J_{C\text{-}F}$ = 8.2125 Hz), 121.4054 (2XCH), 115.01–114.73 (2XCH, $J_{C\text{-}F}$ = 21.03 Hz), 75.18 (1C), 57.23 (2XCH_2), 30.69 (6XCH_3), 30.65 (2XC). MS [EI+] *m/z* (%): 400 $[M]^+$ (20), 262 $[M - C_7H_{12}N_3]^+$ (100). HRMS (EI): for $C_{21}H_{29}FN_6O$ calcd. 400.2387, found 400.2386.

2-Phenyl-1,3-bis-(4-phenyl-[1,2,3]triazol-1-yl)-propan-2-ol (10)

1,3-Diazido-2-phenyl-propan-2-ol and phenylacetylene afforded 2-Phenyl-1,3-bis-(4-phenyl-[1,2,3]triazol-1-yl)-propan-2-ol as a white solid. Yield: 122 mg (58%). IR (ATR) ν_{max} 3570, 3088, 1485 cm^{-1}. ^{1}H NMR: (300 MHz, DMSO-d_6) δ = 8.24 (s, 2H), 7.79 (d, 4H), 7.52–7.42 (m, 11H), 6.37 (s, 1H), 4.89 (d, J = 14.4, 2H), 4.62 (d, J = 14.1 Hz, 2H). ^{13}C NMR: (75 MHz, DMSO-d_6) δ = 146.13 (2XC), 140.74 (C), 131.14 (CH), 129.41 (2XC), 128.42 (2XCH), 128.30 (2XCH), 128.04 (2XCH), 126.31 (2XCH), 125.55 (4xCH), 123.09 (2XCH), 75.30 (C), 57.65 (2XCH_2). MS [EI+] *m/z* (%): 422 $[M]^+$ (11), 264 $[M - C_9H_8N_3]^+$ (100). HRMS (EI): for $C_{25}H_{22}N_6O$ calcd. 422.1855, found 422.1857.

1,3-Bis-(4-cyclopropyl-[1,2,3]triazol-1-yl)-2-phenyl-propan-2-ol (11)

1,3-Diazido-2-phenyl-propan-2-ol and cyclopropylacetylene afforded 1,3-Bis-(4-cyclopropyl-[1,2,3]triazol-1-yl)-2-phenyl-propan-2-ol as a white solid. Yield: 167.2 mg (63%). IR (ATR) ν_{max} 3540, 3110, 1450 cm^{-1}. ^1H NMR: (300 MHz, DMSO-d_6) $\delta = 7.50$ (s, 2H), 7.43–7.30 (m, 5H), 6.19 (s, 1H), 4.76 (d, $J = 13.2$, 2H), 4.59 (d, $J = 13.0$ Hz, 2H), 1.918 (s, 2H), 0.905 (s, 2H), 0.672 (s, 2H). ^{13}C NMR: (75 MHz, DMSO-d_6) $\delta = 148.64$ (2XC), 140.91 (1C), 128.33 (CH), 127.93 (2XCH), 126.28 (2XCH), 122.47 (CH), 75.23 (C), 57.37 ($2XCH_2$), 8.10 ($2XCH_2$), 6.93 ($2XCH_2$). MS [EI+] *m/z* (%): 350 $[M]^+$ (10), 228 $[M - C_6H_8N_3]^+$ (100). HRMS (EI): for $C_{19}H_{22}N_6O$ calcd. 350.1855, found 350.1859.

1,3-Bis-(4-tert-butyl-[1,2,3]triazol-1-yl)-2-phenyl-propan-2-ol (12)

1,3-Diazido-2-phenyl-propan-2-ol and 3,3-Dimethyl-but-1-yne afforded 1,3-Bis-(4-tert-butyl-[1,2,3]triazol-1-yl)-2-phenyl-propan-2-ol as a white solid. Yield: 129.9 mg (57%). IR (ATR) ν_{max} 3516, 3095, 1465 cm^{-1}. ^1H NMR: (300 MHz, DMSO-d_6) $\delta = 7.46$ (s, 2H), 7.36 (m, 2H), 7.26 (m, 3H), 6.32 (s, 1H), 4.77 (d, $J = 14.1$, 2H), 4.59 (d, $J = 14.1$ Hz, 2H), 1.22 (s, 18H). ^{13}C NMR: (75 MHz, DMSO-d_6) $\delta = 155.90$ (2XC), 141.02 (C), 128.20 (CH), 127.82 (2XCH), 126.28 (2XCH), 121.41 (2XCH), 75.33 (C), 57.24 ($2XCH_2$), 33.49 (C), 30.67 ($6XCH_3$). MS [EI+] *m/z* (%): 382 $[M]^+$ (10), 244 $[M - C_7H_{12}N_3]^+$ (100), 77 $[C_6H_5]^+$ (70). HRMS (EI): for $C_{21}H_{30}N_6O$ calcd. 382.2481, found 382.2487.

2-(4-Methoxyphenyl)-1,3-bis-(4-phenyl-[1,2,3]triazol-1-yl)-propan-2-ol (13)

1,3-Diazido-2-(4-methoxy-phenyl)-propan-2-ol and phenylacetylene afforded 2-(4-Methoxy-phenyl)-1,3-bis-(4-phenyl-[1,2,3]triazol-1-yl)-propan-2-ol as white solid. Yield: 133.1 mg (52%). IR (ATR) ν_{max} 3514, 3034, 1431 cm^{-1}. ^1H NMR: (300 MHz, DMSO-d_6) $\delta = 8.30$ (s, 2H), 7.83 (d, $J = 6.9$ Hz, 4H), 7.46 (d, $J = 6.3$ Hz, 4H), 7.37 (d, 6.6 Hz, 2H), 7.36 (m, 2H), 6.88 (d, $J = 7.5$ Hz, 2H), 6.31 (s, 1H), 4.93 (d, $J = 14.6$ Hz, 2H), 4.87 (d, $J = 14.2$ Hz, 2H) 3.74 (s, 3H). ^{13}C NMR: (75 MHz, DMSO-d_6) $\delta = 158.95$ (C), 146.15 (2XC), 132.60 (C), 131.21 (2XC), 129.43 (4XCH), 128.32 (4XCH), 127.60 (2XCH), 125.59 (2XCH), 123.11 (2XCH), 113.73 (2XCH), 75.07 (C), 57.78 ($2XCH_2$), 55.46 (CH_3). MS [EI+] *m/z* (%): 252 $[M]^+$ (20), 294 $[M - C_9H_8N_3]^+$ (100). HRMS (EI): for $C_{26}H_{24}N_6O_2$ calcd. 452.1961, found 452.1965.

1,3-Bis-(4-cyclopropyl-[1,2,3]triazol-1-yl)-2-(4-methoxyphenyl)-propan-2-ol (14)

1,3-Diazido-2-(4-methoxy-phenyl)-propan-2-ol and cyclopropylacetylene afforded 1,3-Bis-(4-cyclopropyl-[1,2,3]triazol-1-yl)-2-(4-methoxy-phenyl)-propan-2-ol as white solid. Yield: 145.8 mg (54%). IR (ATR) ν_{max} 3520, 3067, 1432 cm^{-1}. ^1H NMR: (300 MHz, DMSO-d_6) $\delta = 7.49$ (s, 2H), 7.31 (d, $J = 8.1$ Hz, 2H), 6.85 (d, $J = 8.1$ Hz, 2H), 6.09 (s, 1H), 4.69 (d, $J = 14.1$, 2H), 4.56 (d, $J = 14.0$ Hz, 2H), 3.75 (s, 3H), 1.90 (q, 2H), 0.88 (d, 4H), 0.66 (d, 4H). ^{13}C NMR: (75 MHz, DMSO-d_6) $\delta = 158.85$ (C), 148.60 (2XC), 132.72 (C), 127.5 (2XCH), 122.45 (2XCH), 113.61 (2XCH), 74.94 (C), 55.45 (CH_3) 54.43 ($2XCH_2$), 6.92 (2XCH), 8.09 ($4XCH_2$). MS [EI+] *m/z* (%): 300 $[M]^+$ (10), 258 $[M - C_6H_8N_3]^+$ (100). HRMS (EI): for $C_{20}H_{24}N_6O_2$ calcd. 380.1961, found 380.1966.

1,3-Bis-(4-tert-butyl-[1,2,3]triazol-1-yl)-2-(4-methoxyphenyl)-propan-2-ol (15)

1,3-Diazido-2-(4-methoxy-phenyl)-propan-2-ol and 3,3-Dimethyl-but-1-yne afforded 1,3-Bis-(4-tert-butyl-[1,2,3]triazol-1-yl)-2-(4-methoxy-phenyl)-propan-2-ol as white solid. Yield: 145.8 mg (66%). IR (ATR) ν_{max} 3526, 3067, 1432 cm^{-1}. ^1H NMR: (300 MHz, DMSO-d_6) $\delta = 7.52$ (s, 2H), 7.35 (d, 2H), 6.92 (d, 2H), 6.16 (s, 1H), 4.79 (d, $J = 12.3$, 2H), 4.60 (d, $J = 12.5$ Hz, 2H), 1.31 (s, 18H), 3.81 (s, 3H). ^{13}C NMR: (75 MHz, DMSO-d_6) $\delta = 159.01$ (C), 156.02 (2XC), 132.96 (C), 127.57 (2XCH), 121.50 (2XCH), 113.62 (2XCH), 75.12 (C), 55.57 (CH_3), 57.42 ($2XCH_2$), 33.49 (C), 30.77 ($6XCH_3$). MS [EI +] *m/z* (%): 412 $[M]^+$ (20), 274 $[M - C_7H_{12}N_3]^+$ (100). HRMS (EI): for $C_{22}H_{32}N_6O_2$ calcd. 412.2587, found 412.2589.

2-(4-Chlorophenyl)-1,3-bis-(4-phenyl-[1,2,3]triazol-1-yl)-propan-2-ol (16)

2-{4-[1-(Toluene-4-sulfonyl)-1,2,3-triazol-4-yl]butyl}isoindole-1,3-dione afforded 2-[4-(1,2,3-triazol-4-yl)butyl] isoindole-1,3-dione as white solid. Yield: 145.8 mg (84%). IR (ATR) ν_{max} 3126, 3067, 2940, 2871, 1702 cm^{-1}. ^1H NMR: (300 MHz, $CDCl_3$) $\delta = 13.14$ (s, 1H), 7.84 (dd, $J = 5.4$, 3.0 Hz, 2H), 7.71 (dd, $J = 5.3$, 3.0 Hz, 2H), 7.52 (s, 1H), 3.73 (s, 2H), 2.81 (s, 2H), 1.82 – 1.70 (m, 4H). ^{13}C NMR: (75 MHz, $CDCl_3$) $\delta = 167.35$(2xC), 144.58 (C), 132.78 (2xCH), 130.84 (2xC), 129.70 (CH), 122.06 (2xCH), 36.32 (CH_2), 26.82 (CH_2), 25.20 (CH_2), 23.15 (CH_2). MS [EI+] *m/z* (%): 456 $[M]^+$ (30), 345 $[M - C_6H_4Cl]^+$ (100). HRMS (EI): for $C_{25}H_{21}ClN_6O$ calcd. 456.1465, found 270.1469.

2-(4-Chlorophenyl)-1,3-bis-(4-cyclopropyl-[1,2,3]triazol-1-yl)-propan-2-ol (17)

2-{4-[1-(Toluene-4-sulfonyl)-1,2,3-triazol-4-yl]butyl}isoindole-1,3-dione afforded 2-[4-(1,2,3-triazol-4-yl)butyl]isoindole-1,3-dione as white solid. Yield: 145.8 mg (80%). IR (ATR) ν_{max} 3126, 3067, 2940, 2871, 1702 cm^{-1}. ^1H NMR: (300 MHz, $CDCl_3$) $\delta = 13.14$ (s, 1H), 7.84 (dd, $J = 5.4$, 3.0 Hz, 2H), 7.71 (dd, $J = 5.3$, 3.0 Hz, 2H), 7.52 (s, 1H), 3.73 (s, 2H), 2.81 (s, 2H), 1.82–1.70 (m, 4H). ^{13}C NMR: (75 MHz, $CDCl_3$) $\delta = 167.35$(2xC), 144.58 (C), 132.78 (2xCH), 130.84 (2xC), 129.70 (CH), 122.06 (2xCH), 36.32 (CH_2), 26.82 (CH_2), 25.20 (CH_2), 23.15 (CH_2). MS [EI+] *m/z* (%): 384 $[M]^+$ (10), 273 $[M - C_6H_4Cl]^+$ (100). HRMS (EI): for $C_{19}H_{21Cl}N_6O$ calcd. 384.1465, found 384.1467.

1,3-Bis-(4-tert-butyl-[1,2,3]triazol-1-yl)-2-(4-chlorophenyl)-propan-2-ol (18)

2-{4-[1-(Toluene-4-sulfonyl)-1,2,3-triazol-4-yl]butyl}isoindole-1,3-dione afforded 2-[4-(1,2,3-triazol-4-yl)butyl]isoindole-1,3-dione as white solid. Yield: 145.8 mg (78%). IR (ATR) ν_{max} 3129, 3067, 2940, 2875, 1702 cm^{-1}. ^1H NMR: (300 MHz, $CDCl_3$) $\delta = 13.14$ (s, 1H), 7.84 (dd, $J = 5.4$, 3.0 Hz, 2H), 7.71 (dd, $J = 5.3$, 3.0 Hz, 2H), 7.52 (s, 1H), 3.73 (s, 2H), 2.81 (s, 2H), 1.82–1.70 (m, 4H). ^{13}C NMR: (75 MHz, $CDCl_3$) $\delta = 167.35$(2xC), 144.58 (C), 132.78 (2xCH), 130.84 (2xC), 129.70 (CH), 122.06 (2xCH), 36.32 (CH_2), 26.82 (CH_2), 25.20 (CH_2), 23.15 (CH_2). MS [EI+] *m/z* (%): 270 $[M]^+$ (40), 104 $[C_7H_4O]^+$ (100), 76 $[C_6H_4]^+$ (80). HRMS (EI): for $C_{21}H_{29}ClN_6O$ calcd. 416.2091, found 416.2097.

In vitro antifungal activities

The antifungal activity of compounds **7**–18 was screened using a reference strain of yeasts from American Type Culture Collection, *Candida albicans* ATCC 10231, which was included as a control strain, and four clinical isolates, which were obtained from the Maternal Perinatal Hospital Monica Pretelini Sáenz as well as the following strains provided by the microbiology laboratory of the Universidad Autónoma del Estado de México, these are clinical isolates of infections associated with health care: *Candida krusei*, *Candida glabrata*, and *Candida tropicalis*.

The MICs of compounds **7**–**18** and the control antifungal agents were determined consistently using the broth microdilution method for yeasts developed by the Clinical and Laboratory Standards Institute and published in the document M27-A3 (Rex et al. 2008). For all organisms, RPMI 1640 medium with 0.165 M morpholinepropanesulfonic acid (MOPS) buffer (pH 7.0) was used as the test medium. MICs were determined after incubation at 30 °C for 16 to 24 h and defined by the criteria of the CLSI procedures.

Toxicity evaluation of compounds using *Artemia salina*

The toxicity evaluation was done with the crustacean *Artemia salina* and the procedure was established and standardized as follows: Brine shrimp (*Artemia salina* Leach) eggs were purchased from a pet store. The brine shrimp eggs were hatched in shallow rectangular tray (22 × 23 cm) containing artificial seawater (3.8% sea salt solution, prepared from Instant Ocean Salt Mix, Aquatic Eco-Systems, Inc., FL) at room temperature. The eggs were sprinkled into the darken compartment and after the eggs hatched into free-swimming forms, the nauplii moved to the light side. After 48 h the phototrophic nauplii were collected. A suspension of 10 nauplii was added to each well and the covered microplate was incubated for 24 h at room temperature. The number of dead nauplii in each well was counted using a binocular microscope. For the test, salt water solution was used as positive control, whereas a potassium dichromate was used as negative control. The LD_{50} was determined according to the Reed–Muench method (Sam 1993; Reed and Muench 1938).

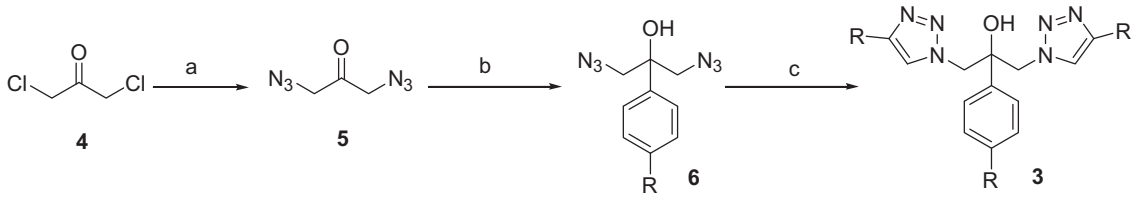
Molecular modeling

All compounds were optimized with the PBE0/3-21G method implemented in the software NWChem 6.5. In each case, was verified that the resulting structure belonged to a minimum of energy on the surface of potential energy, by means of the criterion of non-imaginary frequencies. The docking calculation was performed in the active site reported in the X-ray structure of the sterol 14-alpha demethylase (CYP51) of *Candida albicans* available in the Protein Data Bank, employing autodock vina 1.1.2. With the aim of perform a quantitative description of the electronic properties, some quantum chemical descriptors: Ionization Potential (IP), Electron affinity (EA), electrophilia (χ), hardness (η), and electronic chemical potential (μ) were obtained under the Khon-Sham framework and the PBE0/3-21G//PBE0/3-21++G method. Virtual Computational Chemistry Laboratory at website http://www.vcclab.org was used for the calculation of log P and Log S values of the compounds by various methods based on different theoretical procedures.

Results and discussion

Chemistry

Preliminary studies were focused to prepare 1,3-bis-(1,2,3-triazol-1-yl)-propan-2-ol derivatives **3**, which were obtained through a synthetic sequence that began with SN2 reaction



Scheme 2 Reagents and conditions: **a** NaN_3, acetone. **b** RC_6H_4MgBr, THF-CH_2Cl^2, -75 °C. **c** Alkyne, CuI, NaOH, MeOH

on 1,3-dichloroacetone **4** to afford 1,3-diazidoacetone **5**, which in turn was reacted with diverse Grignard reagents yielding the corresponding 1,3-diazido-2-arylpropan-2-ol derivatives **6** (Scheme 2). In the final step, CuAAC reaction of compounds **6** with the appropriate alkynes gave 1,3-bis-(1,2,3-triazol-1-yl)-propan-2-ol derivatives **3** in ca 50–90% overall yields. These results are summarized in Table 1, showing yields in last CuAAC reaction step.

Biological evaluation

All compounds were characterized by the conventional spectroscopic techniques and compounds **7**–**18** were tested for the in vitro antifungal activity, showing activity against clinical strains of *Candida spp*. isolated from Maternal Perinatal Hospital Monica Pretelini Sáenz and strains provided by the Laboratory of Microbiology of UAEM, as well as control strain *Candida albicans* ATCC 10231. The minimum inhibitory concentrations (MIC) of compounds **7**–**18** against the above mentioned yeast strains are shown in Table 2, according to the microdilution method M27-A3 for yeasts described by CLSI standard protocol (Rex et al. 2008).

From in vitro evaluation, compounds **7**, **9**, **10**, **12**, **13**, **15**, **16**, and **17** displayed inhibition of *Candida albicans*, *Candida krusei* at a concentration of 0.04 µg/mL, and inhibition of *Candida glabrata* and *Candida tropicalis* as well as control strain *Candida albicans* ATCC 10231 at a concentration of 0.5 µg/mL. Hence, MIC of these 1,3-bis-(1,2,3-triazol-1-yl)-propan-2-ol derivatives for *Candida albicans* is lower than other reported for similar compounds with an activity below fluconazole and itraconazole (Salake et al. 2014).

On the other hand, compounds **8** and **14** proved to be effective for the inhibition of strains of *Candida spp*. and the control strain *Candida albicans* ATCC 10231 showing similar behavior to azoles used as control (fluconazole and itraconazole) and compared to other similar compounds (Layton-Tovar et al. 2014; Miyazaki et al. 2011).

A noteworthy feature is that compound **8** exhibits an inhibitory effect on strains of *Candida albicans* and *Candida krusei* below 0.0075 µg/mL, whereas antifungal activity against other Candida strains is similar, and in some cases, lower (*Candida glabrata*, *Candida tropicalis*, MIC = 2.56 µg/mL), which can be attributed to a combination of the effect of cyclopropyl groups and fluorine atom presents in this molecule. This property suggests a high selectivity of compound **8** inhibiting these kind of *Candida* strains.

Table 1 Synthesis of 1,3-bis-(1,2,3-triazol-1-yl)-propan-2-ol derivatives

Compound	R^1	R^2	% yield
7	F	Ph	90
8	F	Cyclopropyl	61
9	F	$(CH_3)_3C$	63
10	H	Ph	58
11	H	Cyclopropyl	63
12	H	(CH3)3C	57
13	CH_3O	Ph	52
14	CH_3O	Cyclopropyl	54
15	CH_3O	$(CH_3)_3C$	66
16	Cl	Ph	84
17	Cl	Cyclopropyl	80
18	Cl	$(CH_3)_3C$	78

Table 2 In vitro antifungal activities of synthesized compounds MIC (µg/mL)

Compound	*Candida albicans ATCC 10231*	*Candida albicans*	*Candida krusei*	*Candida glabrata*	*Candida tropicalis*
7	0.04	0.04	0.04	0.5	0.5
8	<0.0075	<0.0075	<0.0075	2.56	2.56
9	0.04	0.04	0.04	0.5	0.5
10	0.04	0.04	0.04	0.5	0.5
11	0.04	0.04	0.04	0.5	0.5
12	0.04	0.04	0.04	0.5	0.5
13	0.04	0.04	0.04	0.5	0.5
14	0.03	0.04	0.04	0.08	0.08
15	0.04	0.04	0.04	0.5	0.5
16	0.04	0.04	0.04	0.5	0.5
17	0.04	0.04	0.04	0.08	0.08
18	0.04	0.04	0.04	0.08	0.08
Itraconazole	2.56	2.56	2.56	2.56	1.28
Fluconazole	1.28	1.28	2.56	0.128	0.06

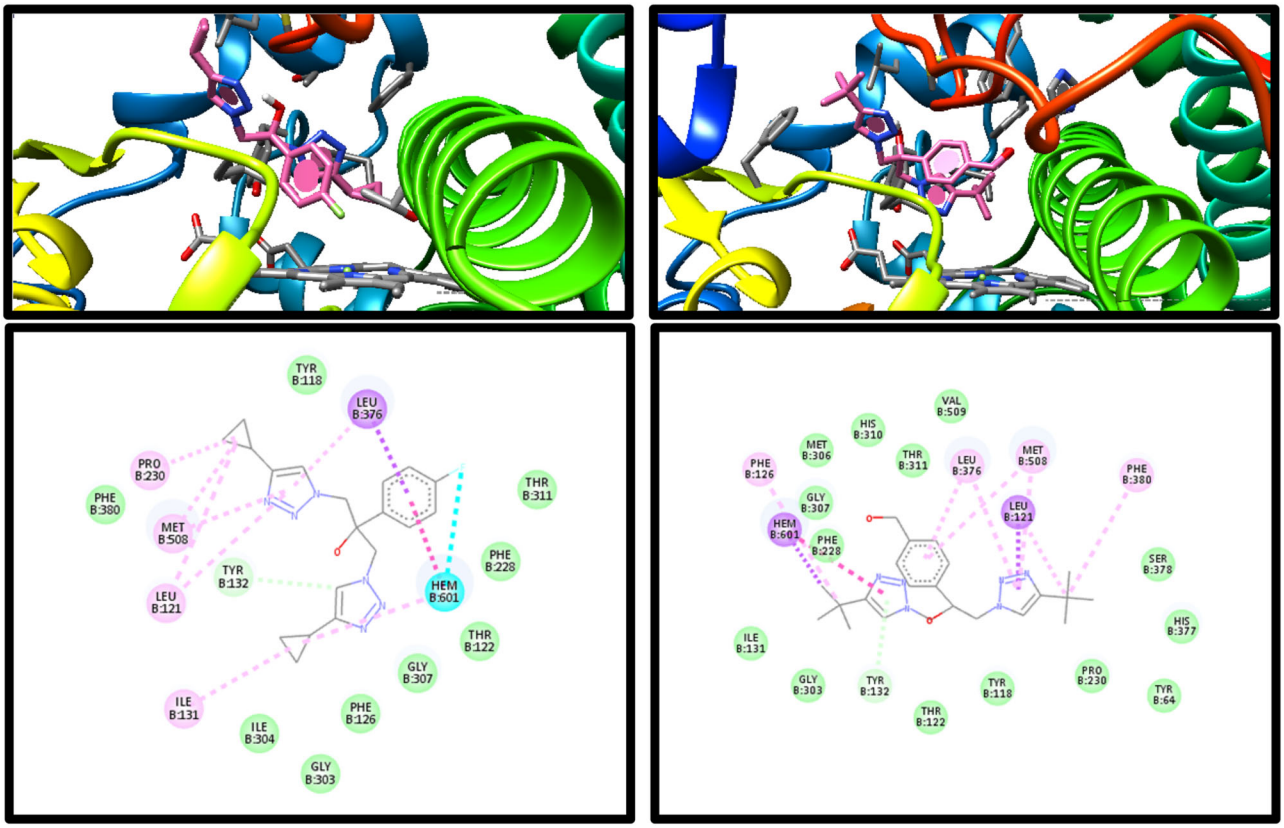


Fig. 2 Docking results and interactions for compounds with best (left) and worstbiological activity (right) on protein 14-alpha demethylase (CYP51)

Furthermore, *Artemia salina* bioassay was used to assess toxicity of compounds **7–18** according to procedures described by the Wiwat (Woradulayapinij et al. 2005) and Camper (Turker and Camper 2002) groups, and corresponding LD_{50} values of the 1,3-bis-(1,2,3-triazol-1-yl)-propan-2-ol derivatives were calculated by Reed-Muench statistical method (Sam 1993; Reed and Muench 1938). From this analysis, which included Fluconazole, Itraconazole, DMSO, and RPMI 1640 with MOPS, together with the controls Saltwater (negative) and potassium dichromate (positive), only potassium dichromate (positive control) and DMSO were toxic to *Artemia salina*, and 1,3-bis-(1,2,3-triazol-1-yl)-propan-2-ol derivatives **7–18** proved to be non-toxic in this assay, as well as RPMI 1640 broth supplemented with MOPS, itraconazole, and fluconazole showed no toxicity to *Artemia salina*. Thus, this study also reveals that studied compounds are relatively harmless to environment (Carballo et al. 2002).

Molecular docking studies

Initial theoretical studies included a geometrical optimization. All the molecular optimized structures show a folded conformation among aromatic rings displaying the formation of two possible intramolecular hydrogen bond interactions, the first interaction occurs between the –OH hydrogen with the N3 triazole electron rich zone, and a second interaction between the C5 triazole hydrogen and the oxygen of the tertiary alcohol. These interactions provide some rigidity to the system and an arrangement, which suggests a less interaction of the tertiary alcohol with the receptor. Some molecular optimized structures are represented in Fig. 1.

In addition, Fig. 2 shows the docking calculation results for the 1,3-bis-(1,2,3-triazol-1-yl)-propan-2-ol derivatives with best (compound **8**) and worst (compound **15**) biological activity, in both cases a direct interaction of the heterocyclic rings to HEME group in 14-alpha demethylase (CYP51) was observed. Other important interactions also take place through the halogen atoms 4-substituted in phenyl ring with HEME moiety, and through π–π interactions for the compound **15** with lowest biological activity. This good binding affinity of halogen atoms could be related with the high antifungal activity in compounds **7**, **8**, **9**, **16**, **17**, and **18**, and would explain an improvement in the antifungal activity when fluorine atoms are used. For compound **8**, we observed an additional interaction of 1,2,3-triazole ring with Met508, which in turn interacts with

Table 3 Quantum molecular descriptors and biological activity for the studied systems, obtained under the PBE0/3-21G//PBE0-3-21++G method

Compound	*A*	*IP*	*EA*	χ	η	μ	*BA*
7	−11.1	7.80	0.33	4.07	3.74	6.72	0.04
8	−9.2	8.27	0.10	4.18	4.09	6.52	<0.0075
9	−9.3	8.59	0.10	4.34	4.25	6.32	0.04
10	−10.6	7.72	0.23	3.98	3.75	6.72	0.04
11	−8.8	8.16	−0.01	4.08	4.09	6.41	0.04
12	−9.2	8.49	0.00	4.24	4.25	6.22	0.04
13	−9.9	7.76	0.26	4.01	3.75	6.37	0.04
14	−8.7	8.19	0.02	4.10	4.09	5.14	0.03
15	−8.9	8.46	0.05	4.26	4.21	6.48	2.56
16	−10	7.80	0.35	4.08	3.73	6.82	0.04
17	−8.1	8.26	0.12	4.19	4.07	6.59	0.04
18	−9.2	8.56	0.12	4.34	4.22	6.37	0.04

Table 4 Predicted drug-likeness properties

Compound	MW	Log P	Log S	TPSA	rotb
7	440.48	3.51	−4.39	81.66	7
8	368.42	2.11	−2.90	81.66	7
9	400.50	3.34	−3.71	81.66	7
10	422.49	3.56	−4.04	81.66	7
11	350.43	2.29	−2.92	81.66	7
12	382.51	3.38	−3.38	81.66	7
13	452.52	3.54	−4.18	90.90	8
14	380.45	2.03	−2.83	90.90	8
15	412.54	3.13	−3.56	90.90	8
16	456.94	3.86	−4.39	81.66	7
17	384.87	2.57	−3.14	81.66	7
18	416.96	3.68	−3.77	81.66	7

Log P denotes lipophilicity, log S aqueous solubility

MW molecular weight, *TPSA* topological polar surface area and rotb, rotatable bonds

cyclopropyl group that was also seen to form interactions with Pro230 and Leu121.

The geometric results provided by a scoring function usually are not enough for the elucidation of the enzyme inhibition phenomena, for this reason we analyzed some electronic descriptors, which are gathered in Table 3: Ionization Potential (IP), Electron affinity (EA), electrophilia (χ), hardness (η), and electronic chemical potential (μ). However, the direct correlation between obtained values with experimental data did not provide additional information that could support a plausible explanation. Similarly, no conclusive results have been found from analysis of some physicochemical properties calculations summarized in Table 4 except that an increase in the hydrophilic character of compounds containing cyclopropyl groups has been identified.

To the best of our knowledge, these are the first examples of Fluconazole analogues where all 1,2,4-triazole rings were changed by 1,2,3-triazoles obtaining a new family of compounds with high antifungal activity, which would open a new trend in the design and synthesis of antifungal compounds

Conclusions

In summary, a small library of 1,3-bis-(1,2,3-triazol-1-yl)-propan-2-ol derivatives analogues to Fluconazole was prepared through click chemistry approach as key reaction through a short synthetic sequence, which required three steps using commercially available starting materials. The compounds evaluated in this investigation presented an important activity to inhibit hospital strains of *Candida spp*. and control strain *Candida albicans ATCC 10231*. Moreover, most of these compounds resulted not toxic to *Artemia salina*, which suggests promising applications as drugs. These elements allow to propose 1,1-diaryl-2-(1,2,3)triazol-1-yl-ethanol derivatives to subsequent biological assays in order to consider them as possible drugs. The simplicity of synthetic methods and antifungal activities found in some compounds suggests that this kind of Fluconazole analogues will enjoy a widespread application.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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