DOI: 10.17344/acsi.2019.4992 *Acta Chim. Slov.* **2019,** *66,* 874–887

Scientific paper

Synthesis, Antifungal Evaluation and Molecular Docking Studies of Some Tetrazole Derivatives

Mohammad Hosein Afsarian,[1] Mojtaba Farjam,[2,3,*] Elham Zarenezhad,[2,*] Somayeh Behrouz[4] and Mohammad Navid Soltani Rad[4]

[1] *Department of Medical Mycology & Parasitology, School of Medicine, Fasa University of Medical Sciences, Fasa, Iran*

[2] *Noncommunicable Diseases Research Center, School of Medicine, Fasa University of Medical Sciences, Fasa, Iran*

[3] *Department of Medical pharmacology, school of medicine, Fasa University of medical sciences, Fasa, Iran*

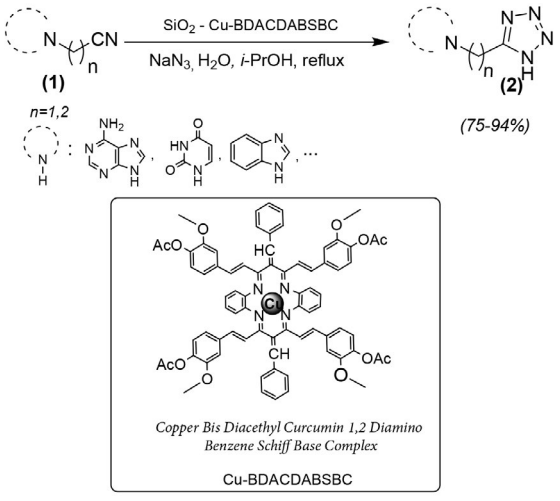
[4] *Medicinal Chemistry Research Laboratory, Department of Chemistry, Shiraz University of Technology, Shiraz, Iran*

* *Corresponding author: E-mail: El.Zarenezhad@gmail.com farjam.md@gmail.com; mfarjam@fums.ac.ir*

Received: 01-25-2019

Abstract

A facile and simple protocol for the [3+2] cycloaddition of alkyl nitriles (RCN) with sodium azide (NaN_3) in the presence of copper bis(diacetylcurcumin) 1,2-diaminobenzene Schiff base complex, SiO_2-[Cu-BDACDABSBC] as a heterogeneous catalyst in the presence of ascorbic acid and a solution of water/*i*-PrOH (50:50, V/V) media at reflux condition is described. The supported catalyst was prepared by immobilization of a copper bis(diacetylcurcumin) 1,2-diaminobenzene Schiff base complex [Cu-BDACDABSBC] on silica gel. The complex has high selectivity, catalytic activity, and recyclability. The significant features of this procedure are high yields, broad substrate scope and simple and efficient work-up procedure. According to this synthetic methodology, excellent yields of 5-substituted 1*H*-tetrazoles having bioactive *N*-heterocyclic cores were synthesized. The *in vitro* antifungal activities of title compounds were screened against various pathogenic fungal strains, such as *Candida* species involving *C. albicans, C. glabrata, C. krusei, C. parapsilosis* as well as filamentous fungi like *Aspergillus* species consisting of *A. fumigatus* and *A. flavus*. The molecular docking analysis is discussed for one most potent compound against fungi. The docking study determined a remarkable interaction between the most potent compounds and the active site of *Mycobacterium* P450DM.



Keywords: 'Click' cycloaddition; tetrazoles; heterogeneous catalyst; antifungal activity; docking studies.

1. Introduction

Tetrazole and its derivatives have attracted considerable interest in recent years because of their unique structure and wide range of applications.[1] Heterocyclic compounds exhibiting tetrazole structures are known as bioactive compounds, encompassing a broad spectrum of biological activities such as antihypertensive,[2] antibacterial,[3,4] antifungal,[5] anticonvulsant,[6] analgesics,[7] anti-inflammatory,[8] antitubercular,[9] anticancer,[10] antineoplastic,[11] antial-

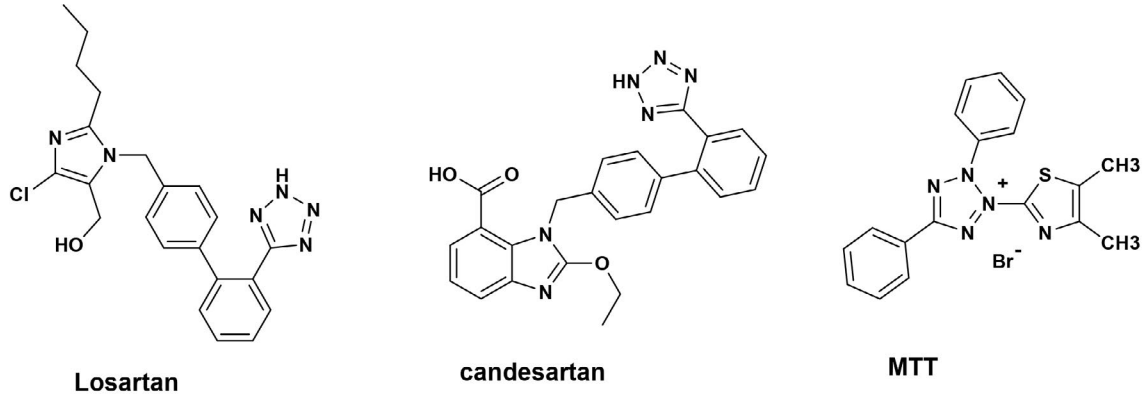


Figure 1. Structure of some of known tetrazol derivatives

lergic,[12] antiviral,[13] and especially anti-HIV activities.[14] They play important roles in coordination chemistry as ligands,[15] in medicinal chemistry as lipophilic spacers and metabolically stable surrogates for the carboxylic acid group and *cis*-amide bond,[16] also in the photographic industry,[17] in agriculture as plant growth regulators.[18] This synthetic heterocyclic nitrogen-rich compounds are also useful synthons in synthetic organic chemistry.[19] In addition, the syntheses of tetrazole moieties are very essential in modern medicinal chemistry, since they can behave as bioisosters for carboxylate moieties. Angiotensin (II) blockers often contain tetrazole cores, as losartan and candesartan.[20] 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromid (MTT) is a well-known tetrazole useful for evaluating cell metabolic activity (Figure 1).[21,22]

The different preparative methods for synthesis of tetrazoles are well documented. The traditional synthesis of 5-substituted 1*H*-tetrazoles is conducted *via* [3+2] cycloaddition of azides to the corresponding nitriles, as developed by Sharpless and coworkers.[23] However, several synthetic approaches have been determined so far that mostly proceed through the non-concerted types of mechanisms.[24] Many homogeneous and heterogeneous catalysts were developed for the synthesis of tetrazoles such as Cu_2O,[25] $AlCl_3$,[26] $BF_3{\cdot}OEt_2$,[27] $Pd(PPh_3)_4$,[28] $Yb(OTf)_3$,[29] $Zn(OTf)_3$,[30] $CuSO_4$,[31] and boron azides,[32] also several heterogeneous catalyst systems, such as silica-supported $FeCl_3$,[33] ZnS nanospheres,[34] zinc hydroxyapatite,[35] Cu–Zn alloy nanopowder,[36] Zn/Al hydrotalcite,[37] Sb_2O_3,[38] metal tungstates,[39] $CdCl_2$,[40] γ-Fe_2O_3,[41] and natural natrolite zeolite[42] were reported.

The copper-based complexes have undeniable roles in 'Click' chemistry; so far various solid compounds were used to support the copper species containing alumina, charcoal, silica gel and zeolites. Recently, the improvement of complexes supported on silica gel has received a great attention,[43] because silica gel as an inorganic support has a high surface area (5–800 $m^2\ kg^{-1}$) compared with other inorganic supports and silica gel consequently ranks at the top of the list of solids with high-surface areas.[44–46] The development of complexes supported on silica gel has received considerable attention, because industry seeks more eco-friendly chemical manufacturing processes.[47–49]

Immobilization of organometallic complexes or homogenous catalysts on inorganic supports seems to be an appropriate approach to improve their stability, reactivity and selectivity. This method obtained the remarkable attention due to the ability of both, to facilitate the catalyst separation and its recycling.[50–56]

The chemical structure of curcumin was discovered by Milobedzka and coworkers. Curcumin as a tautomeric form is diarylheptanoic compound, which represents natural phenols responsible for turmeric's yellow color.[57] The presence of the aromatic ring systems, which are phenols, and connected by two α,β-unsaturated carbonyl groups allows the possibility of grafting with many biomolecules, organic and inorganic materials. Curcumin possesses very interesting pharmacological and biological properties exhibiting a variety of biological activities.[58–59] The heterocyclic N,O-donor Schiff base ligands display a great role in the development of coordination chemistry because they easily form complexes with most of the transition metal ions.[60–61]

The incidence of opportunistic fungal infections has remarkably increased in recent years by normal flora fungi or acquired from the environment, especially *Candida* and *Aspergillus* species in immunocompromised or immunosuppressed patients.[62–64] Increasing of opportunistic pathogenic fungal infections in these patients has become one of the most essential challenges for medicine. To date, it was observed that some of these fungi have become resistant to the established drugs, despite the introduction of new antifungal agents.[65–66] Numerous antifungal drugs are available, such as: azoles (fluconazole, voriconazole and itraconazole) which are considered as the first-line therapy in current clinical use.[67–69] However, the discovery of new antifungal drugs particularly for the treatment of opportunistic pathogenic fungal infections is critically essential.

Since the considerable therapeutic activities of *N*-heterocyclic compounds[70] were demonstrated and also

in continuation of our interest in discovering copper(II) Schiff base complexes[71–72] and the new *N*-heterocyclic bioactive compounds,[73–79] herein we report the application of a suitable and reusable Cu(II)-curcumin complex supported on silica gel and ascorbic acid as the reducing agent. This heterogeneous catalyst system exhibits a potent catalytic activity for the synthesis of some 5-substituated-1*H*-tetrazole derivatives tethered to bioactive *N*-heterocyclic cores.

2. Experimental

2. 1. General

All preliminary chemicals and solvents were purchased from Fluka or Merck. The catalyst was prepared according to the reported procedure.[80] Reactions were monitored by TLC using SILG/UV 254 silica-gel plates. Column chromatography was performed on silica gel 60 (0.063–0.200 mm, 70–230 mesh; ASTM). IR spectra were measured using a Shimadzu FT-IR-8300 spectrophotometer. ^{1}H and ^{13}C NMR spectra were obtained on Bruker Avance-DPX-250/400 spectrometer operating at 250/62.5 and/or 400/100 MHz, respectively. Chemical shifts are given in δ relative to tetramethylsilane (TMS) as the internal standard, coupling constants *J* are given in Hz. Abbreviations used for ^{1}H NMR signals are: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. Elemental analyses were performed on a Perkin–Elmer 240-B micro-analyzer.

2. 2. General Procedure for Immobilization of [Cu-BDACDABSBC] on Silica Gel

To a solution of [Cu-BDACDABSBC] (1.28 g, 1 mmol) in anhydrous dimethyl sulfoxide (55 mL), it was added a fresh and active silica gel (0.6 g, 10 mmol) in 0.063–0.200 mm or 70–230 mesh size, then the mixture was sonicated for 1 h and stirred at room temperature for 48 h. Afterward, the resulting precipitate was filtered off and the solid residue (catalyst) was washed with dimethyl sulfoxide (2 × 50 mL), methanol (3 × 50 mL) and ether (2 × 50 mL), dried in vacuum oven at 60 °C for 4 h and stored in a refrigerator.

2. 3. General Procedure for the Preparation of Alkyl Nitriles 1a–o

To a round bottom flask (100 mL), equipped with a condenser, was added *N*-heterocyclic compound (0.01 mol), 3-chloroacetonitrile or 2-chloropropanenitrile (0.013 mol), K_2CO_3 (0.01 mol), Et_3N (0.01 mol), and a catalytic amount of TBAI (0.1 g) in anhydrous MeCN (40 mL). The reaction mixture was refluxed until TLC monitoring indicated no further progress in the conversion. The solvent was evaporated *in vacuo* to remove the solvent. To continue, the remaining foam was dissolved in $CHCl_3$ (100 mL) and subsequently washed with water (2 × 100 mL). The organic layer was dried (Na_2SO_4) and evaporated. The crude product was purified by column chromatography on silica gel. The catalyst was filtered off, washed with THF/H_2O (5 × 10 mL) and the filtrate was evaporated under vacuum to remove the solvent. The remaining foam was dissolved in $CHCl_3$ (100 mL) and subsequently washed with water (2 × 100 mL). The organic layer was dried (Na_2SO_4) and evaporated. The crude product was purified by column chromatography on silica gel and eluted with proper solvents.

2. 4. General Procedure for the Catalytic Test

In a double-necked round bottom flask (100 mL) equipped with a condenser was added a mixture consisting of alkyl nitrile (0.01 mol), NaN_3 (0.015 mol), and SiO_2-[Cu-BDACDABSBC] (0.05 mol %) in H_2O/*i*-PrOH (1:1 V/V, 50 mL). The mixture was heated at reflux until TLC monitoring indicated no further improvement in the conversion (Table 4). The reaction mixture was then cooled to room temperature, vacuum-filtered and the residue was washed with ethyl acetate (2 × 20 mL). To achieve pH 3, the filtrate was treated with 5 N HCl and stirred at room temperature for 30 minutes. Subsequently, the organic layer was separated, dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel eluted with proper solvents and/or recrystallization was applied. Characterization data of all synthesized compounds are described below.

((1*H*-Tetrazol-5-yl)methyl)-2-methyl-1*H*-benzo[*d*]imidazole (2a)

Recrystallization (EtOAc) afforded a creamy solid; yield: 1.71 g (80%); mp >300 °C (dec.); R_f = 0.25 (EtOAc–MeOH, 1:1); IR (KBr): 3384, 3100, 2982, 1619, 1580, 1480 cm^{-1}; ^{1}H NMR (250 MHz, DMSO-d_6): δ 7.57–7.46 (m, 2H, aryl), 7.16–7.08 (m, 2H, aryl), 5.46 (s, 2H, NCH_2), 4.55 (s, 1H, exchangeable with D_2O, NH, tetrazole), 2.67 (s, 3H, CH_3); ^{13}C NMR (250 MHz, DMSO-d_6): δ 17.90, 49.18, 115.53, 116.64, 121.56, 123.05, 134.72, 140.35, 151.27, 158.41; MS (EI): *m/z* (%) 214 (11.4) [M$^+$]. Anal. Calcd for $C_{10}H_{10}N_6$: C, 56.07; H, 4.71; N, 39.23. Found: C, 56.19; H, 4.62; N, 39.35.

((1*H*-Tetrazol-5-yl)methyl)-1*H*-benzo[*d*]imidazole (2b)

Recrystallization (EtOAc) afforded a yellow solid; yield: 1.80 g (90%); mp 235–240 °C (dec.); R_f = 0.25 (EtOAc–MeOH, 1:1); IR (KBr): 3385, 3100, 2968, 2800, 1616, 1462, 1410 cm^{-1}; ^{1}H NMR (250 MHz, DMSO-d_6): δ 8.28 (s, 1H, C(2)-H, benzimidazole), 7.64–7.61 (m, 2H, aryl), 7.22–7.13 (m, 2H, aryl), 5.55 (s, 2H, NCH_2), 2.51 (s, 1H, exchangeable with D_2O, NH, tetrazole); ^{13}C NMR (250 MHz, DMSO-d_6): δ 51.70, 116.65, 117.85, 122.22, 123.25,

133.61, 137.50, 145.60, 155.69; MS (EI): *m/z* (%) 200 (14.5) [M$^+$]. Anal. Calcd for $C_9H_8N_6$: C, 53.99; H, 4.03; N, 41.98. Found: C, 54.06; H, 4.15; N, 41.92.

((2-Methyl-4-nitro-1*H*-imidazol-1-yl)methyl)-1*H*-tetrazole (2c)

Column chromatography (silica gel, EtOAc–MeOH, 1:1) afforded a brown solid; yield: 1.56 g (75%); mp 208–212 °C (dec.); R_f = 0.31 (EtOAc–MeOH, 1:1); IR (KBr): 3350, 3128, 2900, 1645, 1500, 1456, 1300 cm^{-1}; ^{1}H NMR (250 MHz, DMSO-d_6): δ 8.30 (s, 1H, C(5)-H, imidazole), 5.40 (s, 2H, NCH_2), 4.40 (s, 1H, exchangeable with D_2O, NH, tetrazole), 2.43 (s, 3H, CH_3); ^{13}C NMR (250 MHz, DMSO-d_6): δ 15.74, 48.22, 121.10, 147.82, 153.09, 160.97; MS (EI): *m/z* (%) 209 (8.1) [M$^+$]. Anal. Calcd for $C_6H_7N_7O_2$: C, 34.45; H, 3.37; N, 46.88. Found: C, 34.38; H, 3.42; N, 46.94.

((2-Phenyl-1*H*-imidazol-1-yl)methyl)-1*H*-tetrazole (2d)

Recrystallization (EtOAc) afforded a bright brown solid; yield: 1.92 g (85%); mp 216–220 °C (dec.); R_f = 0.33 (EtOAc–MeOH, 1:1); IR (KBr): 3280, 3150, 2937, 2850, 1653, 1476 cm^{-1}; ^{1}H NMR (250 MHz, DMSO-d_6): δ 7.94–7.91 (m, 2H, aryl), 7.58–7.46 (m, 3H, aryl), 7.20 (s, 1H, C(4)-H, imidazole), 6.95 (s, 1H, C(5)-H, imidazole), 5.28 (s, 2H, NCH_2), 2.50 (s, 1H, exchangeable with D_2O, NH, tetrazole); ^{13}C NMR (250 MHz, DMSO-d_6): δ 49.07, 121.03, 125.54, 127.07, 127.46, 129.68, 131.07, 152.18, 160.14; MS (EI): *m/z* (%) 226 (17.3) [M$^+$]. Anal. Calcd for $C_{11}H_{10}N_6$: C, 58.40; H, 4.46; N, 37.15. Found: C, 58.31; H, 4.58; N, 37.02.

((1*H*-Tetrazol-5-yl)methyl)-1,3-dimethyl-1*H*-purine-2,6(3*H*,7*H*)-dione (2e)

Recrystallization (EtOAc–MeOH) afforded a brown solid; yield: 2.46 g (94%); mp >300 °C (dec.); R_f = 0.27 (EtOAc–MeOH, 1:1); IR (KBr): 3391, 2996, 1720, 1705, 1690, 1650, 1375 cm^{-1}; ^{1}H NMR (250 MHz, DMSO-d_6): δ 8.38 (s, 1H, exchangeable with D_2O, NH, tetrazole), 7.39 (s, 1H, C(8)-H, theophylline), 5.04 (s, 2H, NCH_2), 2.59 (s, 3H, N(1)-CH_3), 2.31(s, 3H, N(3)-CH_3); ^{13}C NMR (250 MHz, DMSO-d_6): δ 27.30, 31.18, 47.34, 104.91, 144.41, 149.32, 151.57, 154.19, 159.10; MS (EI): *m/z* (%) 263 (10.7) [M$^+$]. Anal. Calcd for $C_9H_{10}N_8O_2$: C, 41.22; H, 3.84; N, 42.73. Found: C, 41.28; H, 3.80; N, 42.81.

((1*H*-Tetrazol-5-yl)methyl)pyrimidine-2,4(1*H*,3*H*)-dione (2f)

Recrystallization (EtOAc) afforded a creamy solid; yield: 1.55 g (80%); mp 285–290 °C; R_f = 0.09 (EtOAc–MeOH, 1:1); IR (KBr): 3365, 3129, 2876, 1723, 1706, 1650, 1458 cm^{-1}; ^{1}H NMR (250 MHz, DMSO-d_6): δ 11.37 (s, 1H, exchangeable with D_2O, NH, uracil), 7.67 (d, 1H, *J* = 7.5 Hz, C(6)-H, uracil), 5.70 (d, 1H, *J* = 7.5 Hz, C(5)-H, uracil), 5.07 (s, 2H, NCH_2), 4.07 (s, 1H, exchangeable with D_2O, NH, tetrazole); ^{13}C NMR (250 MHz, DMSO-d_6): δ 47.32, 103.40, 142.15, 151.57, 156.46, 161.72; MS (EI): *m/z* (%) 194 (10.8) [M$^+$]. Anal. Calcd for $C_6H_6N_6O_2$: C, 37.12; H, 3.11; N, 43.29. Found: C, 37.24; H, 3.26; N, 43.24.

((1*H*-Tetrazol-5-yl)methyl)-9*H*-purin-6-amine (2g)

Recrystallization (EtOAc) afforded a creamy solid; yield: 1.71 g (79%); mp >300 °C (dec.); R_f = 0.25 (EtOAc–MeOH, 1:1); IR (KBr): 3328, 3100, 2853, 1676, 1520, 1471 cm^{-1}; ^{1}H NMR (250 MHz, DMSO-d_6): δ 8.12 (s, 1H, C(8)-H, adenine), 8.05 (s, 1H, C(2)-H, adenine), 7.17 (s, 2H, exchangeable with D_2O, NH_2), 5.42 (s, 2H, NCH_2), 4.80 (s, 1H, exchangeable with D_2O, NH, tetrazole); ^{13}C NMR (250 MHz, DMSO-d_6): δ 54.31, 118.43, 139.90, 147.78, 151.57, 155.69, 162.84; MS (EI): *m/z* (%) 217 (9.5) [M$^+$]. Anal. Calcd for $C_7H_7N_9$: C, 38.71; H, 3.25; N, 58.04. Found: C, 38.63; H, 3.18; N, 58.12.

((1*H*-Tetrazol-5-yl)methyl)isoindoline-1,3-dione (2h)

Recrystallization (EtOAc) afforded a creamy solid; yield: 2.08 g (91%); mp 245–249 °C; R_f = 0.47 (EtOAc–MeOH, 1:1); IR (KBr): 3370, 3068, 2981, 1766, 1700, 1660, 1495 cm^{-1}; ^{1}H NMR (250 MHz, DMSO-d_6): δ 7.90–7.85 (m, 4H, aryl), 4.87 (s, 2H, NCH_2), 4.04 (s, 1H, exchangeable with D_2O, NH, tetrazole); ^{13}C NMR (250 MHz, DMSO-d_6): δ 42.20, 127.27, 131.49, 133.48, 157.10, 167.81; MS (EI): *m/z* (%) 229 (15.9) [M$^+$]. Anal. Calcd for $C_{10}H_7N_5O_2$: C, 52.40; H, 3.08; N, 30.56. Found: C, 52.48; H, 3.01; N, 30.69.

((1*H*-Tetrazol-5-yl)methyl)benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide (2i)

Recrystallization (EtOAc) afforded a pale-yellow solid; yield: 2.12 g (80%); mp 225–229 °C (dec.); R_f = 0.18 (EtOAc–MeOH, 1:1); IR (KBr): 3324, 3050, 2976, 1715, 1600, 1460, 1321, 761 cm^{-1}; ^{1}H NMR (250 MHz, DMSO-d$_6$): δ 7.84–7.45 (m, 4H, aryl), 4.44 (s, 2H, NCH_2), 2.51 (s, 1H, exchangeable with D_2O, NH, tetrazole); ^{13}C NMR (250 MHz, DMSO-d_6): δ 39.52, 126.32, 126.88, 127.33, 131.63, 132.06, 139.30, 156.66, 169.10. MS (EI): *m/z* (%) 265 (19.7) [M$^+$]. Anal. Calcd for $C_{10}H_8N_4O_3S$: C, 45.45; H, 3.05; N, 21.20; S, 12.13. Found: C, 45.56; H, 3.11; N, 21.14; S, 12.25.

(2-(2-Methyl-4-nitro-1*H*-imidazol-1-yl)ethyl)-1*H*-tetrazole (2j)

Column chromatography (silica gel, EtOAc–MeOH, 1:1) afforded a creamy solid; yield: 1.74 g (78%); mp 210–215 °C; R_f = 0.23 (EtOAc–MeOH, 1:1); IR (KBr): 3358, 3100, 2965, 1653, 1525, 1460, 1345 cm^{-1}; ^{1}H NMR (250 MHz, DMSO-d_6): δ 8.26 (s, 1H, C(5)-H, imidazole), 4.27 (t, 2H, *J* = 7.2 Hz, NCH_2), 3.61 (s, 1H, exchangeable with D_2O, NH, tetrazole), 3.12 (t, 2H, *J* = 7.2 Hz, NCH_2CH_2), 2.21 (s, 3H, CH_3); ^{13}C NMR (250 MHz, DMSO-d_6): δ 15.99, 27.43, 52.03, 121.49, 148.55, 153.83, 163.97; MS (EI): *m/z* (%) 223 (10.6) [M$^+$]. Anal. Calcd for

$C_9H_{11}N_5O_2$: C, 48.86; H, 5.01; N, 31.66. Found: C, 48.94; H, 5.13; N, 31.59.

5-(2-(2-Phenyl-1*H*-imidazol-1-yl)ethyl)-1*H*-tetrazole (2k)

Recrystallization (EtOAc) afforded a creamy solid; yield: 1.92 g (80%); mp 250–255°C (dec.); R_f = 0.38 (EtOAc–MeOH, 1:1); IR (KBr): 3300, 3050, 2960, 1650, 1485 cm^{-1}; ^1H NMR (250 MHz, DMSO-d_6): δ 7.58–7.44 (m, 5H, aryl), 7.32 (s, 1H, C(4)-H), 6.95 (s, 1H, C(5)-H), 4.29 (t, 2H, *J* = 7.5 Hz, NCH_2), 3.07 (t, 2H, *J* = 7.5 Hz, NCH_2CH_2), 1.98 (s, 1H, exchangeable with D_2O, NH, tetrazole); ^{13}C NMR (250 MHz, DMSO-d_6): δ 30.01, 54.59, 120.83, 125.87, 126.30, 127.05, 127.27, 130.29, 149.77, 155.14; MS (EI): *m/z* (%) 240 (15.8) [M^+]. Anal. Calcd for $C_{12}H_{12}N_6$: C, 59.99; H, 5.03; N, 34.98. Found: C, 60.07; H, 5.16; N, 34.87.

(2-(1*H*-Tetrazol-5-yl)ethyl)-1*H*-benzo[*d*]imidazole (2l)

Recrystallization (EtOAc) afforded a brown solid; yield: 1.79 g (84%); mp 300–304 °C (dec.); R_f = 0.70 (EtOAc–MeOH, 1:1); IR (KBr): 3326, 3100, 2926, 1653, 1501, 1470 cm^{-1}; ^1H NMR (250 MHz, DMSO-d_6): δ 8.10 (s, 1H, C(2)-H, benzimidazole), 7.62–7.55 (m, 2H, aryl), 7.24–7.14 (m, 2H, aryl), 4.55 (t, 2H, *J* = 6.5 Hz, NCH_2), 3.17 (t, 2H, *J* = 6.5 Hz, NCH_2CH_2), 2.51 (s, 1H, exchangeable with D_2O, NH, tetrazole); ^{13}C NMR (250 MHz, DMSO-d_6): δ 31.15, 60.59, 116.19, 117.33, 124.12, 125.22, 134.64, 138.76, 149.31, 161.72; MS (EI): *m/z* (%) 214 (12.7) [M^+]. Anal. Calcd for $C_{10}H_{10}N_6$: C, 55.94; H, 4.63; N, 39.14. Found: C, 55.83; H, 4.75; N, 39.28.

2-(2-(1*H*-Tetrazol-5-yl)ethyl)isoindoline-1,3-dione (2m)

Recrystallization (EtOAc) afforded a creamy solid; yield: 2.11 g (87%); mp >300 °C (dec.); R_f = 0.70 (EtOAc–MeOH, 1:1); IR (KBr): 3374, 3063, 2950, 1772, 1620, 1510, 1458 cm^{-1}; ^1H NMR (250 MHz, DMSO-d_6): δ 7.28–7.09 (m, 4H, aryl), 4.34 (t, 2H, *J* = 7.2 Hz, NCH_2), 3.46 (s, 1H, exchangeable with D_2O, NH, tetrazole), 3.08 (t, 2H, *J* = 7.2 Hz, NCH_2CH_2); ^{13}C NMR (250 MHz, DMSO-d_6): δ 27.64, 42.69, 126.35, 131.64, 134.28, 159.48, 167.02; MS (EI): *m/z* (%) 243 (13.9) [M^+]. Anal. Calcd for $C_{11}H_9N_5O_2$: C, 54.32; H, 3.73; N, 28.79. Found: C, 54.43; H, 3.82; N, 28.86.

1-(2-(1*H*-Tetrazol-5-yl)ethyl)-4-phenylpiperazine (2n)

Column chromatography (silica gel, EtOAc–*n*-hexane, 1:1) afforded a bright brown solid; yield: 2.35 g (91%); mp >300 °C (dec.); R_f = 0.23 (EtOAc–MeOH, 1:1); IR (KBr): 3340, 3100, 2992, 1659, 1653, 1476 cm^{-1}; ^1H NMR (250 MHz, DMSO-d_6): δ 7.26–7.20 (m, 2H, aryl), 6.91–6.81 (m, 3H, aryl), 4.96 (s, 1H, exchangeable with D_2O, NH, tetrazole), 3.83 (t, 2H, *J* = 6.5 Hz, CH_2), 3.09 (t, 2H, *J* = 7.2 Hz, CH_2), 2.72–2.65 (m, 8H, 4 CH_2); ^{13}C NMR (250 MHz, DMSO-d_6): δ 28.22, 49.15, 52.27, 56.11, 114.32, 119.58, 130.51, 150.05, 160.23; MS (EI): *m/z* (%) 258 (19.7) [M^+]. Anal. Calcd for $C_{13}H_{18}N_6$: C, 60.44; H, 7.02; N, 32.53. Found: C, 60.31; H, 7.08; N, 32.61.

7-(2-(1*H*-Tetrazol-5-yl)ethyl)-1,3-dimethyl-1*H*-purine-2,6(3*H*,7*H*)-dione (2o)

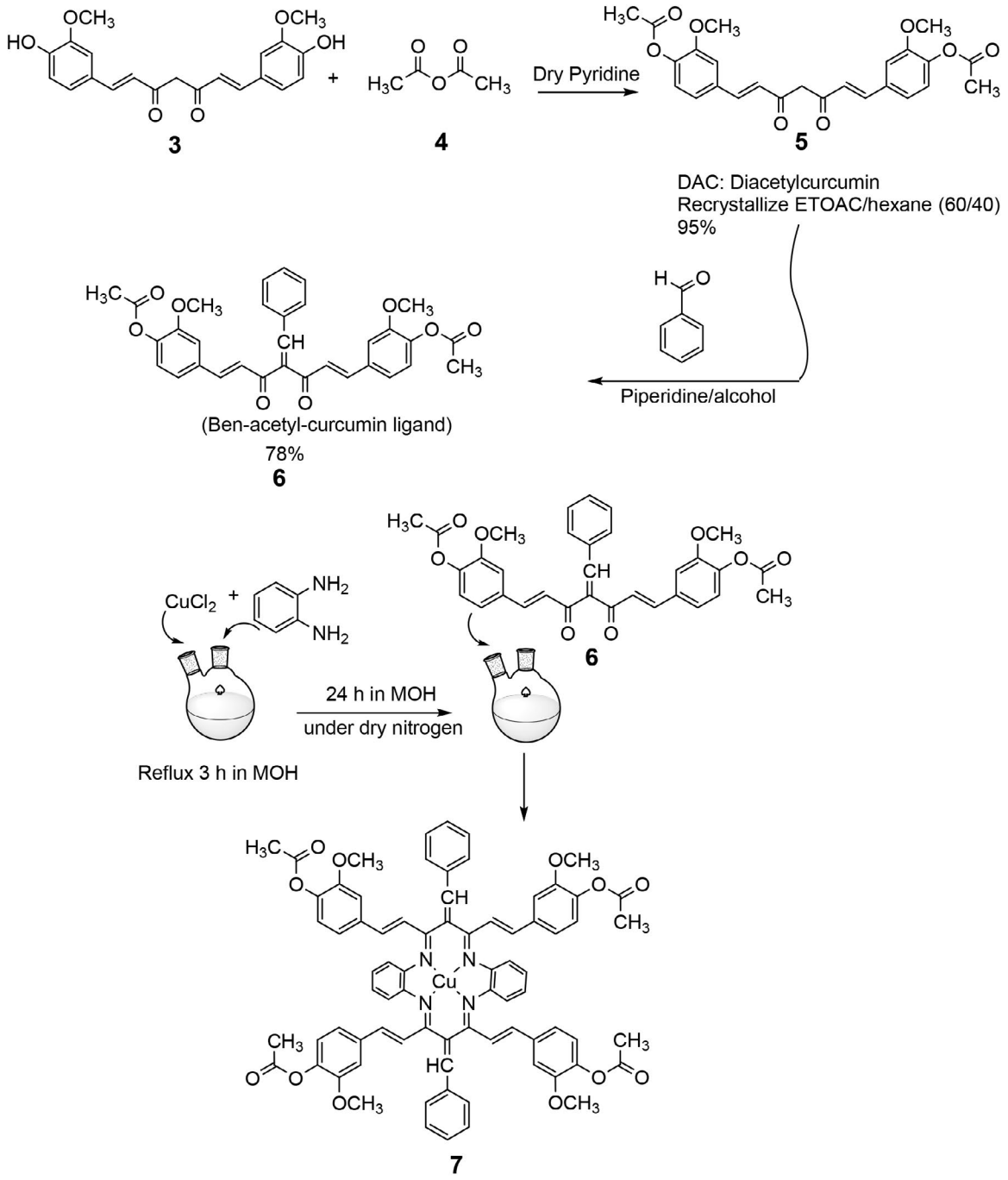
Column chromate-graphy (silica gel, MeOH) afforded a brown foam; yield: 2.34 g (85%); R_f = 0.15 (EtOAc–MeOH, 1:1); IR (KBr): 3300, 2985, 1716, 1702, 1693, 1658, 1379 cm^{-1}; ^1H NMR (250 MHz, DMSO-d_6): δ 7.86 (s, 1H, C(8)-H, theophylline), 4.55 (t, 2H, *J* = 5.7 Hz, NCH_2), 3.41 (t, 2H, *J* = 5.7 Hz, NCH_2CH_2), 3.27 (s, 3H, N(1)-CH_3), 3.09 (s, 3H, N(3)-CH_3), 2.54 (s, 1H, exchangeable with D_2O, NH, tetrazole); ^{13}C NMR (250 MHz, DMSO-d_6): δ 20.17, 31.08, 32.38, 34.54, 106.39, 136.89, 145.91, 147.79, 154.58, 159.86; MS (EI): *m/z* (%) 276 (20.7) [M+]. Anal. Calcd for $C_{10}H_{12}N_8O_2$: C, 43.48; H, 4.38; N, 40.56. Found: C, 43.59; H, 4.27; N, 40.61.

3. Results and Discussion

3. 1. Chemistry of Synthesized Compound

Initially, we prepared an active catalyst according to the procedure reported in the literature[80] as shown in Scheme 1. First, curcumin (**3**) and acetic anhydride (**4**) were stirred in dry pyridine, the yellow crude product was collected and recrystallized from EtOAc/*n*-hexane (60/40) to give diacetylcurcumin (DAC (**5**)) in 95% yield. Then, DAC (452 mg, 1 mmol) and benzaldehyde (108 mg, 1 mmol) were dissolved in alcoholic media in the presence of a catalytic amount of piperidine and which readily afforded Ben-acetyl-curcumin ligand (**6**) in a good yield (76%). Afterwards, for preparation of $Cu^{(II)}$ complex **7**, in a conical flask, copper(II) chloride (1 mmol) and orthophenylenediamine (109 mL, 1 mmol) was refluxed in methanol for about 3 h. To the above solution, benzilidene-acetyl curcumin (574 mg, 1 mmol) (**6**) in methanol was added and the contents were stirred for 24 hour. The microcrystalline product formed, after filtering, the crude was washed with methanol and dried *in vacuo*. After synthesis and supporting the catalyst on silica gel, we applied this heterogeneous catalyst in synthesis of some 1,2,3-triazolyl carbocyclic nucleoside derivatives.

In this methodology, the diverse *N*-heterocycles including imides, xanthines, azoles, purine and pyrimidine nucleobases react with 3-chloropropanenitrile or 2-chloroacetonitrile, in the presence of an equimolar mixture of triethylamine and potassium carbonate (TEA)-K_2CO_3 as the base, and catalytic amount of tetrabutylammonium iodide (TBAI) in acetonitrile at reflux conditions to afford nitriles **1**. In continuation, these nitriles were able to perform the [3+2] cycloaddition reaction with sodium azide using SiO_2-[Cu-BDACDABSBC] as the catalyst, and ascorbic acid as the reducing agent, in water-isopropanol media at reflux condition to yield tetrazoles **2**. In most cas-

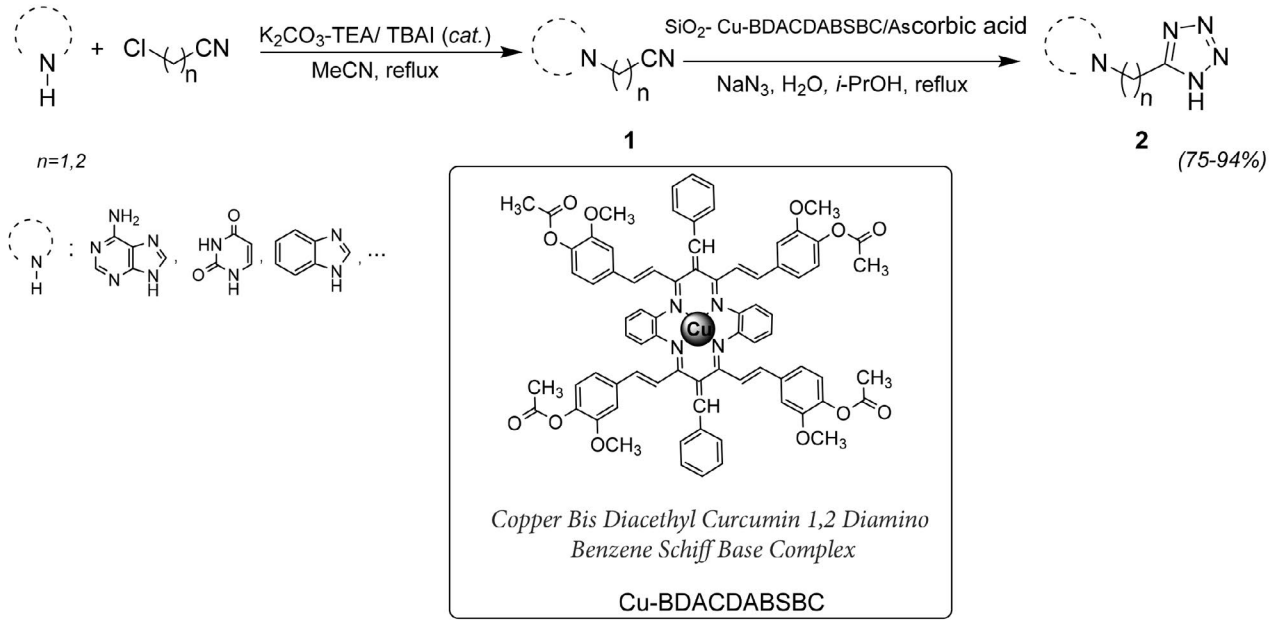


Scheme 1. Preparation of SiO_2-[Cu-BDACDABSBC].

es, the *N*-alkylation reactions were completed after refluxing for 48 h (Scheme 2).

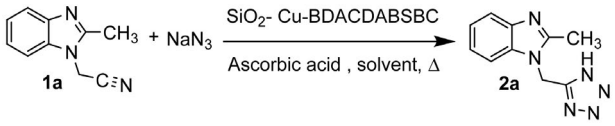
The first step of this synthetic approach was represented by optimization of the reaction conditions. At first, we carried out the cycloaddition reaction of 2-(2-methyl-1*H*-benzo[*d*]imidazol-1-yl) acetonitrile (**1a**) and sodium azide as the model reaction to afford the 1-((1*H*-tetrazol-5-yl)methyl)-2-methyl-1*H*-benzo[*d*]imidazole (**2a**) (Table 1). The 1,3-dipolar cycloaddition of the model reaction was carried out in the presence of ascorbic acid (1 mmol) and SiO_2-Cu-BDACDABSBC (0.05 mol %) in H_2O at different temperatures, which afforded **2a** in 51% yield (as the best result) after refluxing for 5 h (Table 1, entry 6).

To study the influence of temperature and H_2O, the model reaction was carried out at different temperatures (Table 1, entries 2–6). Due to Table 1, an increase in the temperature resulted in the promotion of cycloaddition reaction. The best result was obtained when the cycloaddition reaction was conducted at 100 °C for 5 h (Table 1, entry 6).



Scheme 2. Synthesis of 5-substituated-1*H*-tetrazole derivatives tethered to bioactive *N*-heterocyclic cores using SiO_2-[Cu-BDACDABSBC] and ascorbic acid.

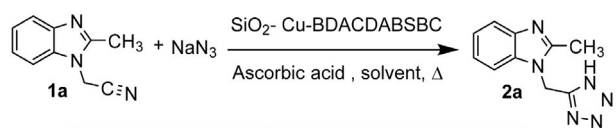
Table 1. Effect of H_2O and temperature on [3+2] cycloaddition reaction of azide-nitrile using Cu-BDACDABSBC and ascorbic acid to afford **2a**[a].



Entry	Solvent	T °C	Time (h)	Yield [b] (%)
1	H_2O	R.T.	24	10
2	H_2O	50	10	20
3	H_2O	60	8	39
4	H_2O	70	8	38
5	H_2O	80	6	49
6	H_2O	Reflux	5	51

[a] Reaction conditions: nitrile (0.01 mol), NaN_3 (0.015 mol), catalyst (0.05 mol %), ascorbic acid (1 mmol), H_2O(50 mL). [b] Isolated yield.

Table 2. Effect of solvent type and temperature on [3+2] cycloaddition reaction of azide-nitrile using Cu-BDACDABSBC and ascorbic acid to afford **2a**[a].



Entry	Solvent	T °C	Time (h)	Yield [b] (%)
1	H_2O/i-PrOH [c]	R.T.	24	60
2	H_2O/i-PrOH [c]	Reflux	4	94
3	H_2O/i-PrOH [d]	Reflux	6	83
4	H_2O/i-PrOH [e]	Reflux	4	65
5	H_2O/Me_2CO [c]	Reflux	4	36
6	H_2O/DMF [c]	Reflux	7	40
7	H_2O/DMSO [c]	Reflux	8	42
8	H_2O/THF [c]	Reflux	8	60
9	H_2O/HMPA [c]	Reflux	6	40
10	H_2O/NMP [c]	Reflux	5	52
11	H_2O/Toluene[c, f]	Reflux	24	45
12	DMSO	120	24	56
13	DMF	120	24	58
14	THF	Reflux	11	50
15	i-PrOH	Reflux	7	52
16	EtOH	Reflux	5	45

[a] Reaction conditions: nitrile (0.01 mol), NaN_3 (0.015 mol), SiO_2-Cu-BDACDABSBC (0.05 mol %), solvent (50 mL). [b] Isolated yield. [c] 50:50 (V/V). [d] 70:30 (V/V). [e] 30:70 (V/V). [f] In the presence of a catalytic amount (0.16 g, 0.0005 mol) of tetrabutylammonium bromide (TBAB)

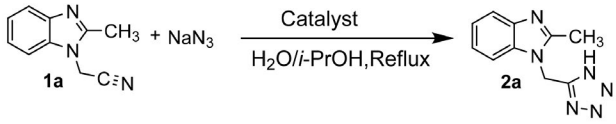
To further optimize the reaction conditions, the influence of various organic solvents/H_2O (V/V) was examined in the presence of SiO_2-Cu-BDACDABSBC (0.05 mol %) and ascorbic acid at various temperature (Table 2).

From Table 2 it is well demonstrated that the solvent has a significant role in the progress of the reaction. Among the examined solvents, a mixture of *i*-PrOH and water (Table 2, entry 1) afforded the best result, compared to pure water (Table 1, entry 6), The best ratio of *i*-PrOH to water for the progress of the reaction was observed to be 1 : 1 (Table 2, entry 2); however other ratios also yielded the product, albeit in lower amounts (Table 2, entries 3, 4). Employing the other mixtures of solvents afforded a moderate yield of the product over longer periods of time (Table 2, entries 5–11). Additionally, when pure aprotic solvents such as DMSO, DMF, THF (Table 2, entries 12–14) and *i*-PrOH and EtOH as protic solvents were used

alone, moderate yields were obtained (Table 2, entries 15–16).

To investigate the catalytic potency of heterogeneous SiO_2-Cu-BDACDABSBC catalyst and other reported copper catalysts in cycloaddition reactions of azide-nitrile, the comparative results are summarized in Table 3. As shown in Table 3, when the reaction was carried out in the absence of a catalyst, this resulted in only marginal yield as indicated by GC analysis (<%7), even if the reaction time was prolonged (Table 3, entry 1).

Table 3. Comparing the catalytic potency of SiO_2-Cu-BDACDABSBC with various catalysts.[a]



Entry	Catalyst	Time (h)	Yield [b] (%)	Ref.
1	–	72	<8	–
2	Cu_2O	10	64	[25]
3	$AlCl_3$	10	76	[26]
4	$FeCl_3$–SiO_2	24	75	[33]
5	This catalyst	4	94	–

[a] Reaction conditions: nitrile (0.01 mol), NaN_3 (0.015 mol), catalyst, H_2O/*i*-PrOH (50 mL). [b] Isolated yield. [c] 0.05 mol %.

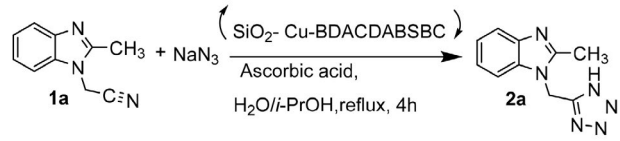
The potency of different reported heterogeneous or homogeneous catalysts in tetrazole synthesis was assessed (Table 3, entries 2–4). As shown in Table 3, higher yield of **2a** were obtained and shorter reaction times were necessary when using heterogeneous SiO_2-Cu-BDACDABSBC catalyst (Table 3, entry 5).

To illustrate the scope of this method, we extended the optimized reaction condition to the cycloaddition reaction of nitrile **1a** with sodium azide (Table 4). As the results in Table 4 indicate, heterogeneous SiO_2-Cu-BDACDABSBC catalyst proved to be useful catalyst for Huisgen cycloaddition between the structurally diverse β-azido alcohols and alkynes. All synthesized compounds **2a**–**o** were fully characterized, and their structures were confirmed by ^1H and ^{13}C NMR spectroscopy, elemental analysis, mass spectrometry and IR spectroscopy methods.

The reusability and recoverability of the SiO_2-Cu-BDACDABSBC catalyst on the sample reaction was studied during the synthesis of **2a** (Table 5). In this connection, prior to the use and also final testing of the catalyst for indicating its activity in many subsequent runs, the catalyst was recycled from the reaction mixture through a sintered glass funnel (vacuum-filtering). The catalyst was washed successively with THF or acetone (10 mL) and dried in a vacuum oven at 80 °C for 30 min.

The catalyst was tested for five consecutive runs and through each run, no fresh catalyst was added. Furthermore, the ICP analysis has confirmed the reusability of

Table 5. The reusability of SiO_2-Cu-BDACDABSBC in successive runs for the synthesis of **2a**.[a]



Run no. [b]	Time (h)	Yield [c] (%)
1	4	94
2	4	92
3	4.5	91
4	4.5	91
5	5	88

[a] Reaction conditions: nitrile (0.01 mol), NaN_3 (0.015 mol), recovered SiO_2-Cu-BDACDABSBC, H_2O/*i*-PrOH (50 mL). [b] The entry number corresponds to the trial number. [c] Isolated yield.

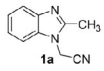
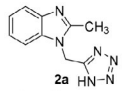
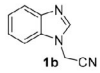
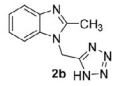
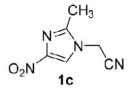
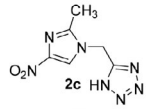
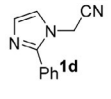
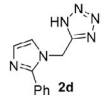
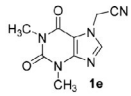
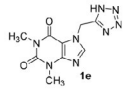
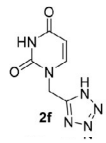
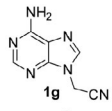
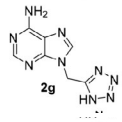
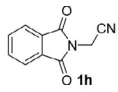
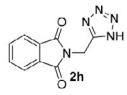
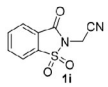
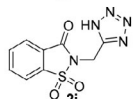
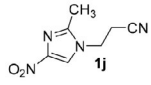
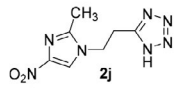
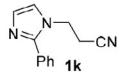
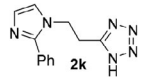
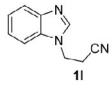
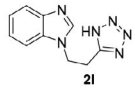
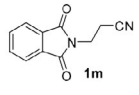
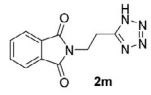
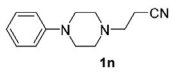
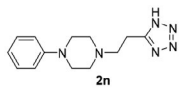
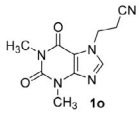
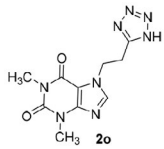
the SiO_2-Cu-BDACDABSBC without significant desorption of Cu species from the silica matrix. As it is well indicated, the amount of leached Cu from SiO_2-Cu-BDACDABSBC is extremely negligible (0.06% after five consecutive runs).

As the results in Table 5 indicate, the catalyst can be reused for many consecutive runs without considerable decrease in its catalytic reactivity.

3. 2. Antifungal Studies

The antifungal activities of 5-substituted-1*H*-tetrazole derivatives against yeasts and filamentous fungi were evaluated *in vitro*. The minimal inhibitory concentrations (MICs) of the tested compounds were determined by the micro broth dilution method in 96-well microplates according to the CLSI-M27-A3 and M27-S4 methods for yeasts[81–82] and CLSI-M38-A2 for filamentous fungi.[83] Antifungal agents as quality controls including amphotericin B (AMB) (Bristol-Myers-Squib, Woerden, The Netherlands), itraconazole (ITZ) (Janssen Research Foundation, Beerse, Belgium), voriconazole (VRZ) (Sigma), posaconazole (PSZ) (Sigma) and fluconazole (FLZ) (Pfizer, Groton, CT, USA) were obtained as reagent-grade powders from the respective manufacturers for the preparation of CLSI microdilution trays. The standard isolates as quality controls were obtained from collections of ATCC (American Type Culture Collection); its yeasts consist of: *Candida* species [*C. albicans* (ATCC 10231), *C. glabrata* (ATCC 2001), *C. krusei* (ATCC 6258), *C. parapsilosis* (ATCC 22019)] and filamentous fungi consist of *Aspergillus* species [*A. fumigatus* (ATCC MYA-2636), *A. flavus* (ATCC 204304)]. Some tetrazol compounds were dissolved in DMSO and serially diluted in the standard RPMI-1640 medium (Sigma Chemical Co.) and buffered to pH 7.0 with 0.165 M-morpholinepropanesulfonic acid (MOPS) buffer (Sigma) and L-glutamine without bicarbonate (maximum concentration was considered 512 μg/mL for

Table 4. The synthesized new 1*H*-tetrazoles using SiO_2-Cu-BDACDABSBC [a]

Entry	Nitrile	Product [b]	Time (h)	Yield [c] (%)
1	 1a	 2a	4	94
2	 1b	 2b	6	90
3	 1c	 2c	6	75
4	 1d	 2d	3	80
5	 1e	 1e	4	95
6	 1f	 2f	3	82
7	 1g	 2g	6	80
8	 1h	 2h	4	91
9	 1i		3	79
10	 1j	 2j	8	79
11	 1k	 2k	7	82
12	 1l	 2l	6	85
13	 1m	 2m	5	85
14	 1n	 2n	6	93
15	 1o	 2o	5	90

[a] Reaction conditions: nitrile (0.01 mol), NaN_3 (0.015 mol), SiO_2-Cu-BDACDABSBC (0.05 mol %), water/*i*-PrOH (50 mL). [b] All products were characterized by ^1H and ^{13}C NMR, IR, CHN, and MS analysis. [c] Isolated yield.

all compounds). Briefly, the inoculum suspension was added to each well and incubated at 35 °C. MIC was defined as the minimum inhibitory concentration of the tested compound which resulted in total inhibition of the fungal growth. All susceptibility testing was performed in duplicate.

Table 6 summarizes the MIC values of 6 standard isolates of yeasts and filamentous fungi of five antifungal drugs and 15 tetrazol compounds. The *in vitro* susceptibility results obtained for FLZ, ITZ, VRZ, PSZ and AMB against the standard isolates were within the ranges that are considered normal for these strains.[84]

The results of MIC of 15 tetrazol derivatives have shown that the lowest MIC with 64 μg/mL was obtained for five compounds (**2g**, **2k**, **2l**, **2m** and **2n**) against *Aspergillus fumigatus* (ATCC MYA-2636), and the highest MIC with 512 and >512 μg/mL was measured for all compounds against *Candida glabrata* (ATCC 2001). In addition, the lowest MIC values against all standard isolates were measured for three tetrazols (**2g**, **2l**, and **2n**), and the highest MIC values against all standard isolates for **2e**. So, Table 6 shows MIC_{50} and MIC_{90} values expressed in μg/mL for all tetrazole derivatives. The lowest MIC_{50} (128 μg/mL) was obtained for **2g**, **2h**, **2i**, **2k**, **2l**, **2m** and **2n** and the highest MIC_{50} (512 μg/mL) for **2e**, thus the lowest MIC_{90} (128 μg/mL) was measured for **2g**, **2l** and **2n** and the highest MIC_{90} (>512 μg/mL) for **2e** and MIC_{90} (512 μg/mL) for **2j** and **2o** (Table 6).

3. 3. Molecular Docking Study

Molecular docking study is a procedure which predicts the interactions of the novel synthetic compound as a drug candidate with the target enzyme and/or receptor binding sites to form a stable complex.[85] Since **2n** was specified as the most potent antifungal agent, thus the binding mode of **2n** in the active site of cytochrome P450-dependent 14 α-lanosterol demethylase was investigated carrying out a molecular docking study.

In the case of docking method, every ligand was optimized with different minimization structures, they were thereafter converted to PDBQT using MGL tools 1.5.6.[86] Co-crystal ligand molecules were excluded from the structures and the PDBs were corrected in terms of missing atom types by modeler 9.12.[87] A house application (MODELFACE) was used for the generation of python script and running modeller software. Consequently, the enzymes were transformed to PDBQT and Gasteiger partial charges were added using MGLTOOLS1.5.6. The docking simulations were achieved by means of an in-house batch script (DOCK-FACE) for automatic running of Auto Dock 4.2,[88] in a parallel mode, using all system resources.

In all experiments genetic algorithm search technique was applied to find the best pose of each ligand in the active site of the target enzyme. Random orientations of the conformations were obtained after translating the center of the ligand to a specified position within the re-

Table 6. *In vitro* susceptibility testing of 6 standard isolates of yeasts and filamentous fungi to four antifungal agents and 15 novel compounds, and MIC_{50} and MIC_{90} values expressed in μg/ml for 5-substituted-1*H*-tetrazole derivatives[a]

Compound	MIC [b] (μg/mL)							
	Candida albicans	*Candida glabrata*	*Candida krusei*	*Candida parapsilosis*	*Aspergillus fumigatus*	*Aspergillus flavus*	MIC_{50}	MIC_{90}
2a	256	512	256	512	128	256	256	256
2b	256	512	256	256	128	256	256	256
2c	256	512	256	256	128	>512	256	256
2d	256	512	256	256	128	>512	256	256
2e	512	>512	512	512	>512	>512	512	>512
2f	256	512	256	256	128	256	256	256
2g	128	256	128	128	64	128	128	128
2h	128	256	128	128	128	256	128	256
2i	256	512	128	256	128	256	128	256
2j	256	512	256	256	256	>512	256	512
2k	128	256	128	128	64	256	128	256
2l	128	256	128	128	64	128	128	128
2m	128	512	256	128	64	256	128	256
2n	128	256	128	128	64	128	128	128
2o	256	512	256	256	>512	256	256	512
Fluconazole [c]	0.25	32	64	0.125	2	2	–	–
Itraconazole [c]	0.25	0.25	0.25	0.063	1	0.5	–	–
Voriconazole [c]	0.125	0.25	0.125	0.031	0. 5	0. 5	–	–
Posaconazole [c]	0.125	0.25	0.125	0.031	0.5	0.25	–	–
Amphotricin B [c]	0.5	0.5	0.5	0.063	1	1	–	–

[a] Examined fungi: *Candida albicans* (ATCC 10231), *C. krusei* (ATCC 6258), *C. glabrata* (ATCC 2001), *C. parapsilosis* (ATCC 22019), *Aspergillus fumigatus* (ATCC MYA-2636) and *A. flavus* (ATCC 204304) [b] Minimal inhibitory concentration [c] Reference drugs for fungal species

ceptor active site, and making a series of rotamers. This process was recursively repeated until the desired number of low energy orientations was obtained. Cluster analysis was performed on the docked results using a root mean square deviation (RMSD) tolerance of 1.8 Å. For the internal validation phase, ligand inside the pdb file of aromatase (1ea1) was extracted using a viewer and treated the same as other ligands in this study.

It is well known that the azoles as antifungal agents are able to inhibit CYP51 through the binding to N-atoms in azoles with the iron core inside the haem. To accredit the docking protocol, fluconazole was redocked in the active site of *Mycobacterium* P450DM (Figure 2). As can be seen, the substrate-binding pocket of *Mycobacterium* P450DM is above the porphyrin ring with the ceiling lipophilic amino acid residues (Phe78, Met79, Phe83, and Phe255). Moreover, access to the pyrrole rings is restricted by Thr260, Ala256, and Leu321, respectively.[89] Just as it was found with fluconazole, compound **2n** is accommodated at the same binding site and showed a strong interaction with *Mycobacterium* P450DM enzyme (Figure 3).

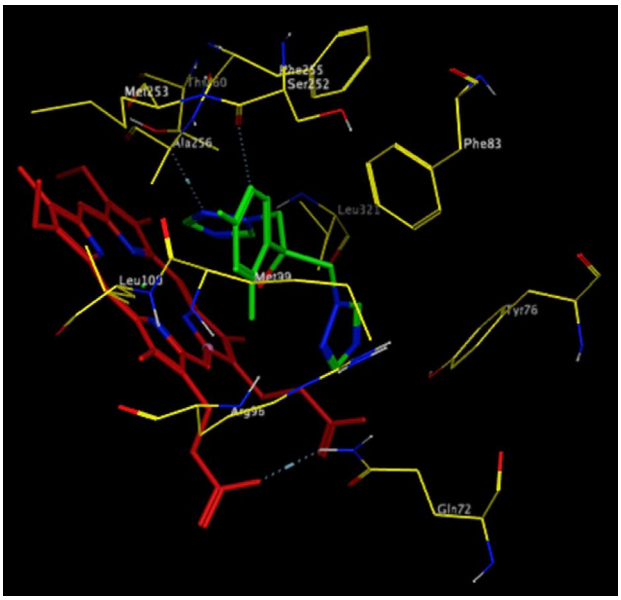


Figure 2. Docking conformation of fluconazole at the active site of *Mycobacterium* P450DM

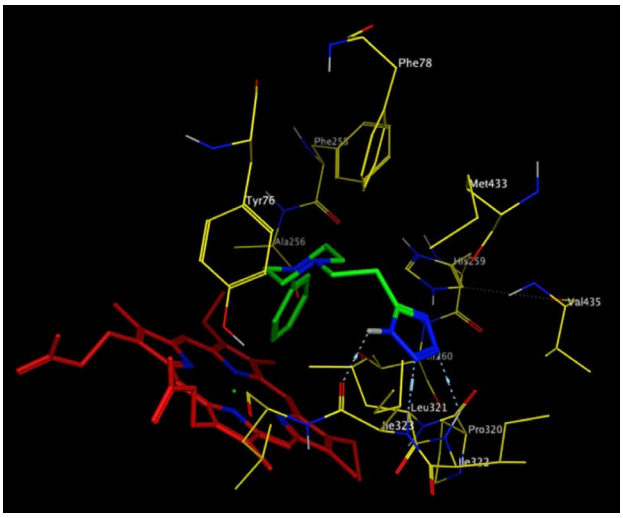


Figure 3. Docking conformation of **2n** at the active site of *Mycobacterium* P450DM

Interestingly, similarly to fluconazole, **2n** is incorporated in the same binding site and showed a strong interaction with the enzyme active site. The calculated bonding energy values for fluconazole and **2n** are –8.10 and –9.22 kcal/mol, respectively, indicating there is an energy gap of about 1.12 kcal/mol. To this energy gap the stronger binding of **2n** at the active site of the enzyme can be attributed. As can be seen in Figure 2, which clearly shows the hydrogen bondings of N^1–H tetrazol with oxygen in Leu324, this hydrogen bonding plays an important role in the higher affinity of **2n** to the active site of the *Mycobacterium* P450DM enzyme. The aliphatic side chain tethered to the tetrazol ring is bound in the hydrophobic pocket above the haem group with residues including Ile323 and Ile 322.

4. Conclusions

In conclusion, we have explained a recyclable heterogeneous catalysts that was used in synthesis of some 5-substituted 1*H*-tetrazoles bearing bioactive *N*-heterocyclic cores. The main advantages of this methodology are its simplicity of the reaction procedure, mild reaction condition, and good to excellent yields. Furthermore, copper(II) catalyst can be recovered and recycled by simple filtration of the reaction mixture and reused for at least five consecutive trials without significant loss of its activity. The antifungal tests have shown antifungal activity against all groups of fungal for some compounds. The docking analysis has demonstrated the appropriate fitting of **2n** in active site of *Mycobacterium* P450DM enzyme.

5. Acknowledgement

We are grateful to Fasa University of Medical Sciences for their financial support.

6. References

1. R. N. Butler, in: A. R. Katritzky, C. W. Rees, E. F. V. Scriven (Ed.): *Comprehensive Heterocyclic Chemistry,* Pergamon, Oxford, **1996**, *4*, pp. 1–126.
2. H. Bräuner-Osborne, J. Egebjerg, E. Ø. Nielsen, U. Madsen, P. Krogsgaard-Larsen, *J. Med. Chem.* **2000**, *43*, 2609–2645. **DOI:**10.1021/jm000007r
3. J. M. Essery, *J. Med. Chem.* **1996**, *12*, 703–705. **DOI:**10.1021/jm00304a039

4. A. Andrus, B. Partridge, J. V. Heck. B. G. Christensen, *Tetrahedron Lett*. **1984**, *25*, 911–1917. **DOI:**10.1016/S0040-4039(01)80060-5
5. V. Dhayanithhi, S. S. Syed, K. Kumaran, K. R. J. Sankar, R. V. Ragavan, P. S. K. Goud, N. S. Kumari, H. N. Pati, *J. Serb. Chem. Soc*. **2011**, *76*, 165–175. **DOI:**10.2298/JSC090421001D
6. R. S. Upadhayaya, S. Jain, N. Sinha, N. Kishore, R. Chandra, S. K. E. Arora, *Eur. J. Med. Chem***. 2004**, *39*, 579–592. **DOI:**10.1016/j.ejmech.2004.03.004
7. A. Rajasekaran, N. Sankar, A. Murugesh, A. R. Kalasalingam, *Arch. Pharm. Res*. **2006**, *29*, 535–540. **DOI:**10.1007/BF02969261
8. P. B. Mohite, R. B. Pandhare, S. G. Khanage, V. H. Bhaskar, *Adv. Pharm. Bull*. **2012**, *2*, 31–36.
9. J. Adamec, K. Waisser, J. Kunes, J. Kaustova, *J. Arch. Pharm*. **2005**, *338*, 385–389. **DOI:**10.1002/ardp.200400967
10. A. O. De Souza, M. T. Pedrosa, J. B. Alderete, A. F. Cruz, M. A. Prado, R. B. Alves, C. L. Silva, *Pharmazie* **2005**, *60*, 396–397.
11. H. Akimoto, K. Ootsu, F. Itoh, *Eur. Patent EP* 530537; *Chem. Abstr*. **1993**, *119*, 226417.
12. G. P. Ellis, D. Shaw, *J. Med. Chem*. **1972**, *15*, 865–867. **DOI:**10.1021/jm00278a027
13. E. Vieira, S. Huwyler, S. Jolidon, F. Knoflach, V. Mutel, J. Wichmann, *J. Bioorg. Med. Chem. Lett*. **2005**, *15*, 4628–4631. **DOI:**10.1016/j.bmcl.2005.05.135
14. A. Gagnon, S. Landry, R. Coulombe, A. Jakalian, I. Guse. B. Thavonekham, P. R. Bonneau, C. Yoakim, B. Simoneau, *Bioorg. Med. Chem. Lett*. **2009**, *19*, 1199–1205. **DOI:**10.1016/j.bmcl.2008.12.074
15. P. N. Gaponik, S. V. Voitekhovich, O. A. Ivashkevich, *Russ. J. Chem. Rev*. **2006**, *75*, 507–539. **DOI:**10.1070/RC2006v075n06ABEH003601
16. R. J. Herr, *Bioorg. Med. Chem*. **2002**, *10*, 3379–3393. **DOI:**10.1016/S0968-0896(02)00239-0
17. G. I. Koldobskii, V. A. Ostrovskii, *Usp. Khim*. **1994**, *63*, 847–865. **DOI:**10.1070/RC1994v063n10ABEH000119
18. J. M. McManus, R. M. Herbst, *J. Org. Chem*. **1959**, *24*, 1464–1467. **DOI:**10.1021/jo01092a021
19. V. Rama, K. Kanagaraj, K. Pitchumani, *J. Org. Chem*. **2011**, *76*, 9090–9095. **DOI:**10.1021/jo201261w
20. V. A. Ostrovskii, R. E. Trifonov, E. A. Popova, *Russ. Chem. Bull.* **2012**, *61*, 768–780. **DOI:**10.1007/s11172-012-0108-4
21. B. Dahlof, R. B. Deveruex, S. E. Kjeldsen, *Lancet* **2002**, *359*, 995–1003. **DOI:**10.1016/S0140-6736(02)08089-3
22. T. Mosmann, *J. Immunol. Methods* **1983**, *65*, 55–63. **DOI:**10.1016/0022-1759(83)90303-4
23. Z. P. Demko, K. B. Sharpless, *Org. Lett*. **2001**, *3*, 4091–4094. **DOI:**10.1021/ol010220x
24. A. R. Katritzky, C. Cai, N. K. Meher, *Synthesis* **2007**, *8*, 1204–1208. **DOI:**10.1055/s-2007-966001
25. T. Jin, F. Kitahara, S. Kamjio, Y. Yamamoto, *Tetrahedron Lett*. **2008**, *49*, 2824–2827. **DOI:**10.1016/j.tetlet.2008.02.115
26. D. P. Matthews, J. E. Green, A. J. Shuker, *J. Comb. Chem*. **2000**, *2*, 19–23. **DOI:**10.1021/cc990035z
27. A. Kumar, R. Narayanan, H. Shechter, *J. Org. Chem*. **1996**, *61*, 4462–4465. **DOI:**10.1021/jo952269k
28. Y. S. Gyoung, J. G. Shim, Y. Yamamoto, *Tetrahedron Lett*. **2000**, *41*, 4193–4196. **DOI:**10.1016/S0040-4039(00)00563-3
29. W. K. Su, Z. Hong, W. G. Shan, X. X. Zhang, *Eur. J. Org. Chem*. **2006**, *12*, 2723–2726. **DOI:**10.1002/ejoc.200600007
30. S. Hajra, D. Sinha, M. Bhowmick, *J. Org. Chem*. **2007**, *72*, 1852–1855. **DOI:**10.1021/jo062432j
31. B. Akhlaghinia, S. Rezazadeh, *J. Braz. Chem. Soc*. 2012, *23*, 2197–2203. **DOI:**10.1590/S0103-50532013005000005
32. Y. Yao, Y. Zhou, B. Lin, C. Yao, *Tetrahedron Lett*. **2013**, *54*, 6779–6781. **DOI:**10.1016/j.tetlet.2013.10.019
33. M. Nasrollahzadeh, Y. Bayat, D. Habibi, S. Moshaee, *Tetrahedron Lett*. **2009**, *50*, 4435–4438. **DOI:**10.1016/j.tetlet.2009.05.048
34. L. Lang, B. Li, W. Liu, L. Jiang, Z. Xu, G. Yin, *Chem. Commun*. **2010**, *46*, 448–450. **DOI:**10.1039/B912284B
35. M. Lakshim Kantam, V. Balasubramanyam, K. B. Shiva Kumar, *Synth. Commun*. **2006**, *36*, 1809–1814. **DOI:**10.1080/00397910600619630
36. G. Aridoss, K. K. Laali, *Eur. J. Org. Chem*. **2011**, *52*, 6343–6355. **DOI:**10.1002/ejoc.201100957
37. M. Lakshmi Kantam, K. B. Shiva Kumar, K. Phani Raja, *J. Mol. Catal. A* **2006**, *247*, 186–188. **DOI:**10.1016/j.molcata.2005.11.046
38. G. Venkateshwarlu, K. C. Rajanna, P. K. Saiprakash, *Synth. Commun*. **2009**, *39*, 426–432. **DOI:**10.1080/00397910802378381
39. J. He, B. Li, F. Chen, Z. Xu, G. Yin, *J. Mol. Catal. A* **2009**, *304*, 135–138. **DOI:**10.1016/j.molcata.2009.01.037
40. G. Venkateshwarlu, A. Premalatha, K. C. Rajanna, P. K. Saiprakash, *Synth. Commun*. **2009**, *39*, 4479–4485. **DOI:**10.1080/00397910902917682
41. G. Qi, Y. Dai, *Chin. Chem. Lett*. **2010**, *21*, 1029–1032. **DOI:**10.1016/j.cclet.2010.05.003
42. M. Nasrollahzadeh, D. Habibi, Z. Shahkarami, Y. Bayat, *Tetrahedron* **2009**, *65*, 10715–10719. **DOI:**10.1016/j.tet.2009.10.029
43. M. N. Soltani Rad, S. Behrouz, M. Doroodmand, A. Movahedian, *Tetrahedron* **2012**, *68*, 7812–7821. **DOI:**10.1016/j.tet.2012.07.032
44. A. Corma, H. Garcia, *Adv. Synth. Catal*. **2006**, *348*, 1391–1412. **DOI:**10.1002/adsc.200606192
45. S. Minakata, M. Komatsu, *Chem. Rev*. **2009**, *109*, 711–724. **DOI:**10.1021/cr8003955
46. C. A. McNamara, M. J. Dixon, M. Bradley, *Chem. Rev.* **2002**, *102*, 3275–3300. **DOI:**10.1021/cr0103571
47. H. Hirai, N. Ohtsuka, T. Shimazawa, *React. Funct. Polym*. **1998**, *37*, 199–212. **DOI:**10.1016/S1381-5148(97)00170-3
48. T. Toupance, M. Kermarec, C. Louis, *J. Phys. Chem. B* **2000**, *104*, 965–972. **DOI:**10.1021/jp993399q
49. N. L. Dias Filho, Y. Gushikem, D. W. Franco, M. S. Schultz, L. C. G. Vasconcellos, *Colloids Surf., A* **1998,** *141*, 181–187. **DOI:**10.1016/S0927-7757(98)00333-1
50. F. Cozzi, *Adv. Synth. Catal*. **2006**, *348*, 1367–1390. **DOI:**10.1002/adsc.200606096
51. K. Binnemans, *Chem. Rev*. **2009**, *109*, 4283–4374. **DOI:**10.1021/cr8003983

52. C. Baleizão, H. Garcia, *Chem. Rev*. **2006**, *106*, 3987–4043. **DOI:**10.1021/cr050973n
53. A. F. Trindade, P. M. P. Gois, C. A. M. Afonso, *Chem. Rev.* **2009**, *109*, 418–514. **DOI:**10.1021/cr800200t
54. J. M. Fraile, J. I. García, J. A. Mayoral, *Chem. Rev.* **2009**, *109*, 360–417. **DOI:**10.1021/cr800363y
55. A. P. Wight, M. E. Davis, *Chem. Rev*. **2002**, *102*, 3589–3614. **DOI:**10.1021/cr020068s
56. L. Yin, J. Liebscher, *Chem. Rev.* **2007**, *107*, 133–173. **DOI:**10.1021/cr0505674
57. J. Miłobędzka, S. von Kostanecki, V. Lampe, *Ber. Dtsch. Chem. Ges*. **1910**, *43*, 2163–2170. **DOI:**10.1002/cber.191004302168
58. R. Waranyoupalin, S. Wongnawa, M. Wongnawa, C. Pakawatchai, P. Panichayupakaranant, P. Sherdshoopongse, *Cent. Eur. J. Chem*. **2009**, *7*, 388–394. **DOI:**10.2478/s11532-009-0037-8
59. A. Goel, A. B. Kunnumakkara, B. B. Aggarwal, *Biochem. Pharmacol*. **2009**, *75*,787–809. **DOI:**10.1016/j.bcp.2007.08.016
60. T. Ueno, M. Ohashi, M. Kono, K. Kondo, A. Suzuki, T. Yamane, Y. Watanabe, *Inorg. Chem*. **2004**, *43*, 2852–2858. **DOI:**10.1021/ic0498539
61. S. Pal, A. K. Barik, S. Gupta, A. Hazra, S. K. Kar, S. M. Peng, G. H. Lee, R. J. Butcher, M. S. E. I. Fallah, J. Ribas, *Inorg. Chem*. **2005**, *44*, 3880–3889. **DOI:**10.1021/ic0501420
62. M. Babazadeh-Qazijahani, H. Badali, H. Irannejad, M. H. Afsarian, S. Emami, *Eur. J. Med. Chem.* **2014**, *76*, 264–273. **DOI:**10.1016/j.ejmech.2014.02.019
63. S. M. H. Afsarian, H. Badali, T. Shokohi, S. Najafipour, *Iran. J. Public Health* **2015**, *44*, 1262.
64. M. H. Afsarian, H. Badali, T. Boekhout, T. Shokohi, F. Katiraee, *J. Med. Microbiol.* **2015**, *64*, 248–253. **DOI:**10.1099/jmm.0.000015
65. S. M. Hashemi, H. Badali, M. A. Faramarzi, N. Samadi, M. H. Afsarian, *Mol. Divers*. **2015**, *19*, 15–27. **DOI:**10.1007/s11030-014-9548-0
66. M. A. Pfaller, *Am. J. Med*. **2012**, 125, 3–13. **DOI:**10.1016/j.amjmed.2011.11.001
67. X. Chai, J. Zhang, Y. Cao, Y. Zou, Q. Wu, D. Zhang, *Eur. J. Med. Chem*. **2012**, *46*, 3142–3148. **DOI:**10.1016/j.ejmech.2011.02.042
68. Y. Y. Zhang, J. L. Mi, C. H. Zhou, X. D. Zhou, *Eur. J. Med. Chem*. **2011**, *46*, 4391–402. **DOI:**10.1016/j.ejmech.2011.07.010
69. T. Shokohi, H. Badali, N. Amirrajab, M. R. Ataollahi, S. A. Kouhpayeh, M. H. Afsarian, *Iran. Curr. Med. Mycol.* **2016**, *2*, 34–39. **DOI:**10.18869/acadpub.cmm.2.2.8
70. M. A. Asif, *J. Bioorg. Chem*. **2017**, *2*, 146–152.
71. S. Esmaielzadeh, E. Zarenezhad, *Acta Chim. Slov*. **2018**, *65*, 416–428. **DOI:**10.17344/acsi.2018.4159
72. E. Zarenezhad, M. N. Soltani Rad, S. Behrouz, S. Esmaielzadeh, M. Farjam, *J. Iran. Chem. Soc.* **2017**, *14*, 509–519. **DOI:**10.1007/s13738-016-0999-3
73. S. Behrouz, M. N. Soltani Rad, S. Rostami, M. Behrouz, E. Zarehnezhad, A. Zarehnezhad, *Mol. Divers*. **2014**, *18*, 797–808. **DOI:**10.1007/s11030-014-9539-1
74. M. N. Soltani Rad, S. Behrouz, M. Behrouz, A.Sami, M. Mardkhoshnood, A. Zarenezhad, E. Zarenezhad, *Mol. Divers*. **2016**, *20*, 705–718. **DOI:**10.1007/s11030-016-9678-7
75. M. N. Soltani Rad, S. Behrouz, E. Zarenezhad, M. H. Moslemin, A. Zarenezhad, M. Mardkhoshnood, M. Behrouz, S. Rostami, *Med. Chem. Res*. **2014**, *23*, 3810–3822. **DOI:**10.1007/s00044-014-0967-3
76. M. N. Soltani Rad, S. Behrouz, E. Zarenezhad, N. Kaviani, *J. Iran. Chem. Soc.* **2015**, *12*, 1603–1612. **DOI:**10.1007/s13738-015-0633-9
77. E. Zarenezhad, M. N. Soltani Rad, M. H. Mosslemin, M. Tabatabaee, S. Behrouz, *J. Chem. Res*. **2014**, *38*, 607–610. **DOI:**10.3184/174751914X14115772243815
78. M. H. Mosslemin, E. Zarenezhad, N. Shams, M. N. Soltani Rad, *J. Chem. Res*. **2014**, *38*, 169–171. **DOI:**10.3184/174751914X13917105358323
79. M. N. Soltani Rad, S. Behrouz, V. Sadeghi Dehchenari, S. J. Hoseini, *J. Heterocycl. Chem*. **2017**, *54*, 355–365. **DOI:**10.1002/jhet.2777
80. J. Rajesh, A. Gubendran, G. Rajagopal, P. Athappan, *J. Mol. Struct*. **2012**, *1010*, 169–178. **DOI:**10.1016/j.molstruc.2011.12.002
81. P. A. Wayne: Reference method for broth dilution antifungal susceptibility testing of yeasts, approved standard. CLSI document M27-A2, **2002**.
82. P. Wayne: Clinical and laboratory standards institute. Implementation Guide of POCT for health care providers, **2006**, 1–37.
83. Clinical Institute LS. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi: Approved Standard. *CLSI document M38-A2*, **2008**.
84. A. L. Barry, M. A. Pfaller, S. D. Brown, A. Espinel-Ingroff, M. A. Ghannoum, C. Knapp, R. P. Rennie, J. H. Rex, M. G. Rinaldi, *J. Clin. Microbiol*. **2000**, *38*, 3457–3459.
85. T. Lengauer, M. Rarey, *Curr. Opin. Struct. Biol.* **1996**, *6*, 402–406. **DOI:**10.1016/S0959-440X(96)80061-3
86. G. M. Morris, R. Huey, A. J. Olson: Using autodock for ligand-receptor docking. *Current Protocols in Bioinformatics*. **2008**, *24*, 8–14. **DOI:**10.1002/0471250953.bi0814s24
87. N. Eswar, B. Webb, M. A. Marti-Renom, M. S. Madhusudhan, D. Eramian, M. Y. Shen, U. Pieper, A. Sali, Comparative protein structure modeling using Modeller. *Current Protocols in Bioinformatics*. **2006**, *15*, 5–6. **DOI:**10.1002/0471250953.bi0506s15
88. A. Sakhteman: PreAuposSOM, *https:// www.biomedicale.univ-paris5.fr/aupossom/*.
89. L. M. Podust, T. L. Poulos, M. R. Waterman, *Proc. Natl. Acad. Sci. U. S. A.* **2001**, *98*, 3068–3073. **DOI:**10.1073/pnas.061562898

Povzetek

V članku opisujemo enostavno sintezno pot, ki temelji na [3+2] cikloadiciji alkil nitrilov (RCN) z natrijevim azidom (NaN_3) v prisotnosti bakrovega kompleksa z bis(diacetilkurkumin) 1,2-diaminobenzensko Schiffovo bazo, ki je imobiliziran na silikagelu: SiO_2-[Cu-BDACDABSBC], ter igra vlogo heterogenega katalizatorja, in askorbinske kisline v zmesi topil vode in *i*-PrOH (50:50, V/V) pod pogoji refluksa. Katalizator na nosilcu smo pripravili z imobilizacijo bakrovega kompleksa bis(diacetilkurkumin) 1,2-diaminobenzenske Schiffove baze [Cu-BDACDABSBC] na silikagel. Ta kompleks se je izkazal kot visokoselektiven katalizator z veliko aktivnostjo in z dobro možnostjo recikliranja. Glavne odlike opisane sinteze so visoki izkoristki, široka paleta možnih izhodnih spojin ter enostavna in učinkovita izolacija, kar je omogočilo pripravo 5-substituiranih 1*H*-tetrazolov, ki so vključevali *N*-heterociklične bioaktivne sisteme, z odličnimi izkoristki. Za pripravljene spojine smo *in vitro* določili protiglivično učinkovanje na nekatere vrste patogenih gliv iz rodu *Candida* (*C. albicans*, *C. glabrata*, *C. krusei* in *C. parapsilosis*) ter na nekatere filamentne glive iz rodu *Aspergillus* (*A. fumigatus* in *A. flavus*). Študije molekulskega sidranja smo izvedli za najbolj učinkovito izmed pripravljenih spojin, kar nam je omogočilo, da smo razvoljali izjemno interakcijo med to spojino in aktivnim mestom *Mycobacterium* P450DM.