SYNTHESIS AND BIOLOGICAL SCREENING OF SOME NOVEL TRIAZOLE DERIVATIVES

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Abstract

*In the present study 15 novel triazolecompounds were synthesized in order to investigate their anticandidal and anticholinesterase activities. Structures of the synthesized compounds were elucidated by spectral data and elemental analyses. Anticandidalactivity tests were performed against four different fungal strains. Compounds **4j**, **4k**, and **4l** displayed moderateanticandidal activity against Candidaglabrata(ATCC 90030)and Candidaalbicans (ATCC 10231). Anticholinesterase activity of the synthesized compounds against acetylcholinesterase (AChE) was also studied. However synthesized compounds did not indicate important enzyme inhibition.*

Key words: *Triazole, Anticandidal, Anticholinesterase*

Bazı Yeni Triazol Türevlerinin SenteziveBiyolojik AktivilerininİncelenmesI

*Bu çalışmada antikandidal ve antikolinesteraz aktivitelerini incelemek amacıyla 15 yeni triazol türevi bileşik sentezlenmiştir. Sentezi gerçekleştirilen bileşiklerin yapıları spektral veriler ve elementel analiz sonuçları yardımıyla aydınlatılmıştır. Bileşiklerin antikandidal aktiviteleri dört farklı mantar suşuna karşı test edilmiştir. Bileşik **4j**, **4k** ve **4l**Candida glabrata(ATCC 90030)ve Candida albicans'a (ATCC 10231)karşıorta düzeydeantikandidal aktivite göstermiştir. Sentezlenen bileşiklerin asetilkolinesteraza (AChE) karşı antikolinesteraz aktiviteleri de incelenmiştir. Ancak, bileşiklerin hiçbiri önemli bir enzim inhibisyonugöstermemiştir.*

Anahtarkelimeler: *Triazol, Antikandidal, Antikolinesteraz*

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INTRODUCTION

In the past two decades, the incidence of systemic fungal infections has been rising dramatically due to an increasing number of immunocompromised hosts (1). The most frequently implicated risk factors contain treatment with broad-spectrum antibiotics, use of central venous catheters and implantable prosthetic devices, parenteral nutrition, prolonged intensive care unit stay, hemodialysis and immunosuppression including HIV infection, neutropenia, use of glucocorticosteroids, chemotherapeutic agents, and immunomodulators (2).

Candida species have emerged as the most common cause of systemic fungal infections (2). For the treatment of these infections, only four important classes of antifungal agents are available in clinical use. These are azoles such as fluconazole, itraconazole, and ketoconazole; polyene macrolides as Amphotericin B and nystatin; 5-flucytosine and echinocandins as caspofungin and micafungin (3).Among them, azoles are the most widely used antifungal agents on account of their high therapeutic index, broad spectrum of activity and more favorable safety profile (4).

Azole type antifungal drugs has been divided into two groups namely triazoles and imidazoles(5). The most frequently used triazoles are fluconazole and itraconazole that display a wide spectrum of antifungal activity and reduced toxicity when compared with imidazole antifungals (6). Several novel triazole antifungal drugs, such as voriconazole, posaconazole, ravuconazole and albaconazole are available in the market or are at the late stages of clinical trials (7).These antifungal drugs act by competitive inhibition of the lanosterol 14α-demethylase (CYP51A1), which is the key enzyme in sterol biosynthesis of fungi. Selective inhibition of CYP51A1 would cause depletion of ergosterol, a major component of the fungal cell membrane, and accumulation of lanosterol and other 14-methyl sterols resulting in the growth inhibition of fungal cells (7,8).

1,2,4-triazole and its derivatives represent an interesting class of compounds, which have been explored for their wide spectrum of pharmacological properties as antibacterial, antifungal(9,10),antimycobacterial(11),antitubercular, anti HIV,sedatives, antianxiety(12),CNS depressant, anti-inflammatory(13),anticancer, analgesic, anticonvulsant(14),herbicidal, insecticidal, antihypertensive, hypoglycemic, antiparasitic, and plant growth activities (15-17). It has been reported that 1,2,4-triazole based antifungal drug tebuconazole causes significant inhibition on acetylcholine esterase level (18).

Prompted from the observations above we synthesized a new series of triazole derivatives, and investigated their anticandidal and anticholinesterase activities so as to obtain new biologically active compounds.

EXPERIMENTAL

Chemistry

The chemicals used in syntheses were purchased from Merck (Germany), Acros (Belgium), ABCR (Germany) or Sigma-Aldrich (Germany) companies. Melting points of the compounds were determined in open capillaries on an Electrothermal 9001 Digital Melting Point Apparatus and were uncorrected. IR spectra were recorded on a Shimadzu, 8400 FTIR spectrometer as KBr pellets. ^{1}H-NMR spectra were recorded on a BrukerUltrashield 500 MHz spectrometer in deutero dimethyl sulfoxide.MS data were obtained on an Agilent 1100 Series LC/MSD Trap VL&SL spectrometer. Elemental analyses (C, H, and N) were determined on a Leco CHNS-932 analyser.

General procedure for 4-(1,2,4-Triazol-1-yl)-1-nitrobenzene (1)

4-Fluoro-1-nitrobenzene (4.24 mL, 0.04 mol), K_2CO_3 (5.52g 0.04 mol), 1,2,4-triazole (2.76 g, 0.04 mol), and DMF (10 mL) wereadded into a vial (30mL) of microwave synthesis reactor(Anton-Paar Monowave 300). The reaction mixture, was heated under conditions of 200 ^{o}Cand 10 bar for 15 min. After cooling, the mixture was poured into iced-water, precipitated product was washed with water, dried and recrystallized from ethanol.Yield: 89 %. m.p. 189^{o}C.Literaturem.p. 190-192 ^{o}C(19).

General procedure for 4-(1,2,4-Triazol-1-yl)aniline (2)

4-(1,2,4-Triazole-1-yl)-1-nitrobenzene (**1**) (6.65 g, 0.035 mol) was dissolved in ethanol (100 mL) and 25% HCl(100 mL) mixture. Zinc powder (22.75 g, 0.35 mol) was divided into ten equal portions (2.275 g x 10) and each portion was added to the stirring solution in 15 min intervals. Once the addition of the zinc was completed, reaction mixture was refluxed for 1h. Hot solution was allowed to cool down, poured into ice water and then neutralized by using 10% NaOH solution. The precipitatewas extracted with chloroform (3x100 mL). The extracts were combined and filtered over anhydrous Na_2SO_4. The solvent was evaporated and the residue was recrystallized from ethanol to give the 4-(1,2,4-triazol-1-yl)aniline. Yield: 68 %.m.p. 160^oC. Literaturem.p. 160-162^oC(20).

General procedure for 2-Chloro-N-[4-(1,2,4-Triazol-1-yl)phenyl]acetamide (3)

4-(1,2,4-Triazole-1-yl)aniline(**2**) (3.52 g 0.022 mol) and triethylamine (3.2 ml 0.06 mol) were dissolved in THF (100 mL). This mixture was allowed to stir on an ice bath. Chloroacetylchloride (1.8 ml, 0.022 mol) in THF (10 ml) was added drop by drop. After this stage, the content was stirred for 1h at room temperature. THF was evaporated and the product was recrystallized from ethanol. Yield: 86 % ;mp: 146^oC.^{1}H-NMR (500 MHz) (DMSO-d_6) δ(ppm): 4.34 (2H, s, $COCH_2$), 7.72-9.23 (6H, m, Ar-H), 10.59 (H, s, NHCO) Es-Ms (m/z): M+1:237.7 (13%), M+1+2: 239.7 (4%). Anal. calcd. For $C_{10}H_9ClN_4O$: C, 50.75; H, 3.83; N, 23.67. Found: C, 50.58; H, 3.81; N, 23.74.

General procedure for 2-Substituted-sulfanyl-N-[4-(1,2,4-triazol-1-yl)phenyl]acetamide derivatives (4a-4o)

2-Chloro-N-[4-(1,2,4-triazole-yl)phenyl]acetamide (**3**) (0.24 g, 0.001 mol), potassium carbonate (0.138 g, 0.001 mol) and appropriate mercaptoazole or mercaptobenzazole derivative (0.001 mol) was dissolved in acetone. The solution was refluxed at 40^oC for 12 h. Acetone was evaporated, residue was washed with water, filtered, dried and recrystallized from ethanol.

2-(Benzimidazol-2-yl)sulfanyl-N-[4-(1,2,4-triazol-1-yl)phenyl]acetamide(4a)

Yield: 72%.M.p. 264^oC. IR ν_{max}(cm^{-1}): 2987-2822 (C-H), 1668 (C=O) 1620-1420 (C=C and C=N), 835 (1,4-disubstituted benzene). ^{1}H-NMR (500 MHz) (DMSO-d_6) δ(ppm): 4.31 (2H, s, $COCH_2$), 7.10-9.20 (10H, m, Ar-H), 10.50 (H, s, NHCO), 12.69 (s, NH). Es-Ms (m/z): M+1: 351.4 (20 %). Anal. calcd. For $C_{17}H_{14}N_6OS$: C, 58.27; H, 4.03; N, 23.98. Found: C, 58.08; H, 4.01; N, 23.93.

2-(Benzoxazol-2-yl)sulfanyl-N-[4-(1,2,4-triazol-1-yl)phenyl]acetamide (4b)

Yield: 75%.M.p. 300 ^oC. IR ν_{max}(cm^{-1}): 2987-2901 (C-H), 1629 (C=O), 829 (1,4-disubstitüted benzene). ^{1}H-NMR (500 MHz) (DMSO-d_6) δ(ppm): 4.35 (2H, s, $COCH_2$), 6.90-9.30 (10H, m, Ar-H), 10.40 (H, s, NHCO). Es-Ms (m/z): M+1: 352.5 (24%). Anal. calcd. For $C_{17}H_{13}N_5O_2S$: C, 58.11; H, 3.73; N, 19.93. Found: C, 58.18; H, 3.74; N, 19.95.

2-(Benzothiazol-2-yl)sulfanyl-N-[4-(1,2,4-triazol-1-yl)phenyl]acetamide (4c)

Yield:81%.M.p. 173^oC. IR ν_{max}(cm^{-1}): 3177 (N-H), 2987-2822 (C-H), 1689 (C=O) 1620-1425 (C=C and C=N), 833 (1,4-disubstituted benzene). ^{1}H-NMR (500 MHz) (DMSO-d_6) δ(ppm): 4.44 (2H, s, $COCH_2$), 7.36-9.22 (10H, m, Ar-H), 10.68 (H, s, NHCO). Es-Ms (m/z): M+1: 368.4 (21%). Anal. calcd. For $C_{17}H_{13}N_5OS_2$: C, 55.57; H, 3.57; N, 19.06. Found: C, 55.29; H, 3.55; N, 19.04.

2-(1,2,4-Triazol-5-yl)sulfanyl-N-[4-(1,2,4-triazol-1-yl)phenyl]acetamide(4d)

Yield:69%.M.p. 232^oC. IR ν_{max}(cm^{-1}): 2987-2901 (C-H), 1678 (C=O) 1620-1388 (C=C and C=N), 827 (1,4-disubstituted benzene). ^{1}H-NMR (500 MHz) (DMSO-d_6) δ(ppm): 4.32 (2H, s, $COCH_2$), 7.63-

9.21 (7H, m, Ar-H), 10.50 (H, s, NHCO), 14.10 (H, s, NH). Es-Ms (m/z): M+1: 302.3 (14%). Anal. calcd. For $C_{12}H_{11}N_7OS$: C, 47.83; H, 3.68; N, 32.54. Found: C, 47.59; H, 3.66; N, 32.42.

2-(1-Methyl-1,2,4-triazol-5-yl)sulfanyl-N-[4-(1,2,4-triazol-1-yl)phenyl]acetamide (4e)

Yield: 72%. M.p. 268°C. IR $\nu_{max}(cm^{-1})$: 2987-2970 (C-H), 1668 (C=O) 1620-1393 (C=C and C=N), 827 (1,4-disubstituted benzene). ^{1}H-NMR (500 MHz) (DMSO-d_6) δ (ppm): 3.62 (3H, s, N-CH_3), 4.30 (2H, s, $COCH_2$), 7.73-9.21 (7H, m, Ar-H), 10.52 (H, s, NHCO). Es-Ms (m/z): M+1: 316.4 (17%). Anal. calcd. For $C_{13}H_{13}N_7OS$: C, 49.51; H, 4.16; N, 31.09. Found: C, 48.98; H, 4.16; N, 31.04.

2-(5-Methyl-1,3,4-thiadiazol-2-yl)sulfanyl-N-[4-(1,2,4-triazol-1-yl)phenyl]acetamide (4f)

Yield:76%.M.p. 219°C. IR $\nu_{max}(cm^{-1})$: 2987-2930 (C-H), 1703 (C=O) 1629-1408 (C=C and C=N bonds), 837 (1,4-disubstituted benzene). ^{1}H-NMR (500 MHz) (DMSO-d_6) δ (ppm): 2.68 (3H, s, CH_3), 4.30 (2H, s, $COCH_2$), 7.73-9.21 (6H, m, Ar-H), 10.59 (H, s, NHCO). Es-Ms (m/z): M+1: 333.4 (17%). Anal. calcd. For $C_{13}H_{12}N_6OS_2$: C, 46.97; H, 3.64; N, 25.28. Found: C, 46.81; H, 3.61; N, 25.21.

2-(1-Methylimidazole-2-yl)sulfanyl-N-[4-(1,2,4-triazole-yl)phenyl]acetamide (4g)

Yield: 71%.M.p. 190°C. IR $\nu_{max}(cm^{-1})$: 2987-2901 (C-H), 1692 (C=O) 1620-1406 (C=C and C=N), 829 (1,4-disubstituted benzene). ^{1}H-NMR (500 MHz) (DMSO-d_6) δ(ppm): 3.60 (3H, s, N-CH_3), 3.90 (2H, s, $COCH_2$), 6.97-9.20 (8H, m, Ar-H), 10.58 (H, s, NHCO).Es-Ms (m/z): M+1: 315.3 (15%). Anal. calcd. For $C_{14}H_{14}N_6OS$: C, 53.49; H, 4.49; N, 26.73. Found: C, 53.18; H, 4.47; N, 26.71.

2-(1-Phenyltetrazol-5-yl)sulfanyl-N-[4-(1,2,4-triazol-1-yl)phenyl]acetamide (4h)

Yield: 77%.M.p. 245°C. IR $\nu_{max}(cm^{-1})$: 2972-2901 (C-H), 1676 (C=O) 1620-1410 (C=C and C=N), 825 (1,4-disubstituted benzene). ^{1}H-NMR (500 MHz) (DMSO-d_6) δ (ppm): 4.44 (2H, s, $COCH_2$), 7.23-9.21 (11, m, Ar-H), 10.66 (H, s, NHCO). Es-Ms (m/z): M+1: 379.4 (24%). Anal. calcd. For $C_{17}H_{14}N_8OS$: C, 53.96; H, 3.73; N, 29.61. Found: C, 53.68; H, 3.74; N, 29.62.

2-(1-Methyltetrazol-5-yl)sulfanyl-N-[4-(1,2,4-triazol-1-yl)phenyl]acetamide (4i)

Yield: 68%.M.p. 284°C. IR $\nu_{max}(cm^{-1})$: 2988-2901 (C-H), 1680 (C=O) 1620-1395 (C=C and C=N), 837 (1,4-disubstituted benzene). ^{1}H-NMR (500 MHz) (DMSO-d_6) δ (ppm): 3.99 (3H, s, N-CH_3), 4.32 (2H, s, $COCH_2$), 7.72-9.21 (6H, m, Ar-H), 10.60 (H, s, NHCO). Es-Ms (m/z): M+1: 317.3 (14%). Anal. calcd. For $C_{12}H_{12}N_8OS$: C, 45.56; H, 3.82; N, 35.42. Found: C, 45.39; H, 3.84; N, 35.44.

2-(5-Nitrobenzimidazol-2-yl)sulfanyl-N-[4-(1,2,4-triazol-1-yl)phenyl]acetamide (4j)

Yield: 78%.M.p. 255°C. IR $\nu_{max}(cm^{-1})$: 2988-2901 (C-H), 1697 (C=O) 1612-1408 (C=C and C=N bonds), 824 (1,4-disubstituted benzene). ^{1}H-NMR (500 MHz) (DMSO-d_6) δ (ppm): 4.39 (2H, s, $COCH_2$), 7.60-9.21 (9H, m, Ar-H), 10.70 (H, s, NHCO), 13.40 (H, s, NH). Es-Ms (m/z): M+1: 396.4 (23%). Anal. calcd. For $C_{17}H_{13}N_7O_3S$: C, 51.64; H, 3.31; N, 24.80. Found: C, 51.93; H, 3.32; N, 24.94.

2-(5-Methylbenzimidazol-2-yl)sulfanyl-N-[4-(1,2,4-triazol-1-yl)phenyl](4k)

Yield: 75%. M.p. 256°C. IR $\nu_{max}(cm^{-1})$: 2970-2901 (C-H), 1670 (C=O) 1620-1408 (C=C and C=N), 840 (1,4-disubstituted benzene). ^{1}H-NMR (500 MHz) (DMSO-d_6) δ (ppm): 2.38 (3H, s, CH_3), 4.28 (2H, s, $COCH_2$), 6.94-9.21 (9H, m, Ar-H), 10.74 (H, s, NHCO), 12.53 (H, s, NH).Es-Ms (m/z): M+1:

365.4 (24%). Anal. calcd. For $C_{18}H_{16}N_6OS$: C, 59.32; H, 4.43; N, 23.06. Found: C, 59.63; H, 4.41; N, 23.04.

2-(5-Methylbenzoxazol-2-yl)sulfanyl-N-[4-(1,2,4-triazol-1-yl)phenyl]acetamide (4l)

Yield: 76%.M.p. 180°C. IR $\nu_{max}(cm^{-1})$: 2970-2900 (C-H), 1691 (C=O) 1630-1408 (C=C and C=N), 835 (1,4-disubstituted benzene). ^{1}H-NMR (500 MHz) (DMSO-d_6) δ (ppm): 2.30 (3H, s, CH_3), 4.30 (2H, s, $COCH_2$), 7.10-9.20 (9H, m, Ar-H), 10.70 (H, s, NHCO). Es-Ms (m/z): M+1: 366.3 (100%). Anal. calcd. For $C_{18}H_{15}N_5O_2S$: C, 59.16; H, 4.14; N, 19.17. Found: C, 59.48; H, 4.12; N, 19.16.

2-(5-Chlorobenzimidazol-2-yl)sulfanyl-N-[4-(1,2,4-triazol-1-yl)phenyl]acetamide (4m)

Yield: 77%.M.p. 257°C. IR $\nu_{max}(cm^{-1})$: 2970-2901 (C-H), 1672 (C=O) 1620-1412 (C=C and C=N), 835 (1,4-disubstituted benzene). ^{1}H-NMR (500 MHz) (DMSO-d_6) δ (ppm): 4.32 (2H, s, $COCH_2$), 7.14-9.21 (9H, m, Ar-H), 10.68 (H, s, NHCO). Es-Ms (m/z): M+1:385.9 (21%), M+1+2: 387.9(6%). Anal. calcd. For $C_{17}H_{13}ClN_6OS$: C, 53.06; H, 3.40; N, 21.84. Found: C, 52.87; H, 3.41; N, 21.79.

2-(5-Nitrobenzoxazol-2-yl)sulfanyl-N-[4-(1,2,4-triazol-1-yl)phenyl]acetamide (4n)

Yield: 75%.M.p 134°C. IR $\nu_{max}(cm^{-1})$: 2988-2901 (C-H), 1630 (C=O) 1517-1379 (C=C and C=N), 835 (1,4-disubstituted benzene). ^{1}H-NMR (500 MHz) (DMSO-d_6) δ (ppm): 4.24 (2H, s, $COCH_2$), 6.97-9.35 (9H, m, Ar-H), 10.70 (H, s, NHCO). Es-Ms (m/z): M+1: 397.4 (19%). Anal. calcd. For $C_{17}H_{12}N_6O_4S$: C, 51.51; H, 3.05; N, 21.20. Found: C, 51.86; H, 3.04; N, 21.18.

2-(5-Chlorobenzothiazol-2-yl)sulfanyl-N-[4-(1,2,4-triazole-1-yl)phenyl]acetamide (4o)

Yield: 79%.M.p. 189°C. IR $\nu_{max}(cm^{-1})$: 2988-2901 (C-H), 1657 (C=O) 1543-1408 (C=C and C=N), 835 (1,4-disubstituted benzene). ^{1}H-NMR (500 MHz) (DMSO-d_6) δ (ppm): 4.44 (2H, s, $COCH_2$), 7.42-9.22 (9H, m, Ar-H), 10.68 (H, s, NHCO). Es-Ms (m/z): M+1:402.1 (22%), M+1+2: 404.2 (7%). Anal. calcd. For $C_{17}H_{12}ClN_5OS_2$: C, 50.81; H, 3.01; N, 17.43. Found: C, 51.09; H, 3.03; N, 17.38.

Biological activity screening

Anticandidal assay

Final products were tested for their in vitro growth inhibitory activity against human pathogenic as *Candida albicans(ATCC 10231), Candida krusei(ATCC 6258), Candida parapsilosis (ATCC 7330)*and *Candida glabrata*(*ATCC 90030).* Fluconazole was used as a positive control. Anticandidalactivity test was performed according to CLSI reference M27-A3 broth microdilution method (21) as described in our previous study (22). Twice MIC readings were carried out for each chemical agent. The compounds were dissolved in DMSO. Further dilutions of the compounds and standard drug in test medium were prepared at the required quantities of 800, 400, 200, 100, 50, 25, 12.5, 6.25, 3.125, and 1.5625 l μg/mL concentrations with Mueller–Hinton broth and Sabouroud dextrose broth. In order to ensure that the solvent per se had no effect on yeast growth, a control test was also performed containing inoculated broth supplemented with only DMSO at the same dilutions used in our experiments and found inactive in culture medium.

AChE Inhibition

All compounds were subjected to a slightly modified method of Ellman's test (23,24) in order to evaluate their potency to inhibit the AChE. Donepezil hydrochloride was used as a positive control (Table 2).Enzyme solutions were prepared in gelatin solution (1%), at a concentration of 2.5 units/mL.AChE and compound solution (50 μL) which is prepared in 2% DMSO at 0.1 and 1 mM

concentrations were added to 3.0 mL phosphate buffer (pH 8±0.1) and incubated at 25 ^{o}C for 5 min. The reaction was started by adding DTNB) (50 µL) and ATC (10 µL) to the enzyme-inhibitor mixture. The production of the yellow anion was recorded for 10 min at 412 nm. As a control, an identical solution of the enzyme without the inhibitor is processed following the same protocol. The blank reading contained 3.0 mL buffer, 50 µL 2% DMSO, 50 µL DTNB and 10 µL substrate. All processes were assayed in triplicate. The inhibition rate (%) was calculated by the following equation:

$$\text{Inhibition \%} = [(AC-AB) - (AI-AB)] / (AC-AB) \times 100$$

Where AI is the absorbance in the presence of the inhibitor, AC is the absorbance of the control and AB is the absorbance of blank reading. Both of the values are corrected with blank-reading value. SPSS for Windows 15.0 was used for statistical analysis. Student's t- test was used for all statistical calculations. Data were expressed as Mean ± SDinactive in culture medium.

RESULTS AND DISCUSSION

In the present work, the reaction sequence outlined in Scheme 1 was followed for the synthesis of 2-(substitutedsulfanyl)-N-[4-(1,2,4-triazol-1-yl)phenyl]acetamide derivatives **(4a–4o)**. Initially, microwave supported synthesis of 4-(1,2,4-triazol-1-yl)-1-nitrobenzene **(1)**was performed in DMF. Secondly, reduction of compound **1** by Zn/HCl in EtOH gave the 4-(1,2,4-triazol-1-yl)aniline (**2**). In the third step, compound **2** was acetylated with chloroacetyl chloride to afford 2-chloro-N-[4-(1,2,4-triazol-1-yl)phenyl]acetamide (**3**) as a starting compound. Then the compound **3**in acetone was reacted with appropriate benzazolethiol derivative in thepresence of potassium carbonate to obtain target compounds.The chemical structures of the compounds (**4a–4o**) were confirmed by IR, ^{1}H NMR, and mass spectral data and elemental analyses.Characteristic stretching absorption of C=O groups were observed at 1630-1703 cm^{-1}as expected. The stretching absorption at about 1388-1629 cm^{-1} were recorded for C=C and C=N double bonds respectively. The stretching absorption for 1,4-disubstituted benzene bond at about 829-856 cm^{-1}. In the ^{1}H-NMR spectra, all of the aromatic and aliphatic protons were observed at estimated areas. N-H proton of amide group gave singlet at 9.90-10.75 ppm and -CH_2 protons gave peaks at 4.12-4.47 ppm. The multiplet belonging to aromatic protons of 1,4-disubstituted phenyl and triazole was appeared at 6.9-9.3ppm. The mass spectra (Es-Ms) of compounds showed [M+1] peaks, in agreement with their molecular formula. All compounds gave satisfactory elemental analyses results.

The in vitro anticandidal activity was measured by means of the minimal inhibitory concentrations (MIC) using the serial dilution method against various *Candida* species. The MIC determination was performed according to the Clinical and Laboratory Standards Institute (CLSI) recommendations (21).The MIC values are summarized in **Table 1**. As seen in the table *Candida krusei(ATCC 6258)*and*Candida parapsilosis(ATCC 7330)* showed resistance against to test compounds. None of the compounds showed valuable anticandidal effect against these strains. On the other hand, *Candida glabrata*(*ATCC 90030)* and*Candida albicans(ATCC 10231)*were more sensitive strains to synthesized compounds. The compounds **4j**, **4k**, and **4l**exhibited moderate anticandidal activity (MIC=25 µg/mL) against these *Candida* species. Observed results suggest that 5-nitro or 5-methyl substitution of benzimidazole or benzoxazole substructures enhance the anticandidalactivity.The anticholinesterase effects of the compounds (**4a-4o**) were determined by modified Ellman's spectrophotometric method **Table 2**. Donepezil was used as a standard AChE inhibitor. None of the compounds showed comparable activity with Donepezil and significant anticholinesterase activity contrary to expectations.

CONCLUSION

In conclusion, a series of novel triazole derivatives were synthesized and studied for their anticandidal and anticholinesterase activities. According to the activity test results, compound **4j**, **4k**, and **4l**exhibited the highest anticandidal activity against *Candida glabrata*(*ATCC 90030)*and*Candida albicans(ATCC 10231)*. The results indicate that the 5-nitro or 5-methyl substitution of benzimidazole or benzoxazole substructures have an impact on the anticandidal activity. However, none of the compounds showed AChE inhibitory activity as much as standard drug Donepezil.

REFERENCES

1.Jiang Y, Cao Y, Zhang J,Zou Y, Chai X, Hu H, Zhao Q, Wu Q, Zhang D, Jiang Y, Sun Q, Design, synthesis and antifungal evaluation of 1-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)propyl)-1H-1,2,4-triazol-5(4H)-one, Eur J MedChem 46, 3135-3141, 2011.

2.Altıntop MD, Kaplancıklı ZA, Turan-Zitouni G, Özdemir A, İşcan G, AkalınG,Yıldırım Ş, Synthesis and anticandidal activity of new triazolothiadiazine derivatives,Eur J Med Chem 46, 5562-5566, 2011.

3.Wang W,Wang S, Liu Y, Dong G, Cao Y, Miao Z, Yao J, Zhang W, Sheng C, Novel conformationally restricted triazole derivatives with potent antifungal activity, Eur JMedChem 45,6020-6026, 2010.

4.Jiang Z, Wang Y, Wang W, Wang S, Xu B, Fan G, Dong G, Liu Y, Yao J, Miao Z,Zhang W, Sheng C, Discovery of highly potent triazole antifungal derivatives by heterocycle-benzene bioisosteric replacement,EurJ MedChem 64,16-22, 2013.

5.Stefanska J, Szulczyk D, Koziol AE, Miroslaw B, Kedzierska E, Fidecka S, Busonera B, Sanna G, Giliberti G, La Colla P, Struga M,Disubstitutedthiourea derivatives and their activity on CNS: synthesis and biological evaluation, Eur J MedChem 55, 205-213, 2012.

6. Muralikrishna A, Venkatesh BC, Padmavathi V, Padmaja A, Kondaiah P, Krishna NS,Synthesis, antimicrobial and cytotoxic activities of sulfone linked bisheterocycles,Eur J Med Chem 54, 605-614, 2012.

7. Zoumpoulakis P, Camoutsis CH, Pairas G, Soković M, Glamočlija J, Potamitis C, Pitsas A,Synthesis of novel sulfonamide-1,2,4-triazoles, 1,3,4-thiadiazoles and 1,3,4-oxadiazoles, as potential antibacterial and antifungal agents, biological evaluation and conformational analysis studies,BioorgMedChem 20, 1569-1583, 2012.

8. Kathiravan MK, Salake AB, Chothe AS, Dudhe PB, Watode RP, Mukta MS, Gadhwe S,The biology and chemistry of antifungal agents: a review, Bioorg MedChem 20, 5678-5698, 2012.

9.Karthikeyan MS, Prasad DJ, Poojary B, Bhat KS, Holla BS, Kumari NS, Synthesisandbiologicalactivity of SchiffandMannichbasesbearing 2,4-dichloro-5-fluorophenyl moiety, BioorgMedChem 14, 7482–7489, 2006.

10. Xu J, Cao Y, Zhang J, Yu S, Zou Y, Chai X, Wu Q, Zhang D, Jiang Y, Sun Q, Design, synthesis and antifungal activities of novel 1,2,4-triazole derivatives, Eur J Med Chem 46, 3142-3148. 2011.

11. Ezabadi IR, Camoutsis C, Zoumpoulakis P, Geronikaki A, Sokovic M, Glamocilija J, Ciric A, Sulfonamide-1,2,4-triazole derivatives as antifungalandantibacterialagents: Synthesis, biological evaluation, lipophilicity, andconformationalstudies, BioorgMedChem 16, 1150–1161, 2008.

12. Patel NB, Khan IH, Rajani SD, Pharmacologicalevaluationandcharacterizations of newlysynthesized1,2,4-triazoles, Eur J Med Chem 45, 4293-4299, 2010.

13. Desai NC, Shihora PN, Rajpara KM, Joshi VV, Vaghani HV, Satodiya HM, Dodiya AM, Synthesis, characterization, andantimicrobialevaluation of novelnaphthalene-based1,2,4-triazoles, MedChemRes 21, 2981–2989, 2012.

14.Khanage SG, Mohite PB, Pandhare RB, Raju SA,MicrowaveAssistedSynthesis of 1-[5-(SubstitutedAryl)-1H-Pyrazol-3-yl]-3,5-Diphenyl-1H-1,2,4-Triazole as AntinociceptiveandAntimicrobialAgents, AdvanPharmaBull4, 105-112, 2014.

15. Chohan ZH, Sumrra SH, Some biologically active oxovanadium(IV) complexes of triazole derived Schiff bases: their synthesis, characterization and biological properties, J Enzyme Inhib MedChem 25, 599-607, 2010.

16. Aggarwal N, Kumar R, Dureja P, KhuranaJM,Synthesis, antimicrobial evaluation and QSAR analysis of novel nalidixic acid based 1,2,4-triazole derivatives, Eur J Med Chem 46, 4089-4099, 2011.

17. Mohsen UA,Biological evaluation of some triazole and triazolothiadiazine derivatives, Mar Pharm J 16, 229-234, 2012.

18. Kolesarova V, Sinko G, Sivikova K, Dianovsky J,In vitro inhibition of blood cholinesterase activities from cattle by triazole fungicides,Caryologia 66, 346-350, 2013.

19. Yang K, Qiu Y, Li Z, Wang Z, Jiang S,Ligands for copper-catalyzed C-N bond forming reactions with 1 molCuBr as catalyst, J Org Chem 76, 3151-3159, 2011.

20. Fun HK, ChingKhengQuah,a, B. Chandrakantha,bArun M. Isloor,c and Prakash Shettyd 4-(1,2,4-Triazol-1-yl)aniline, ActaCryst E67, o164, 2011.

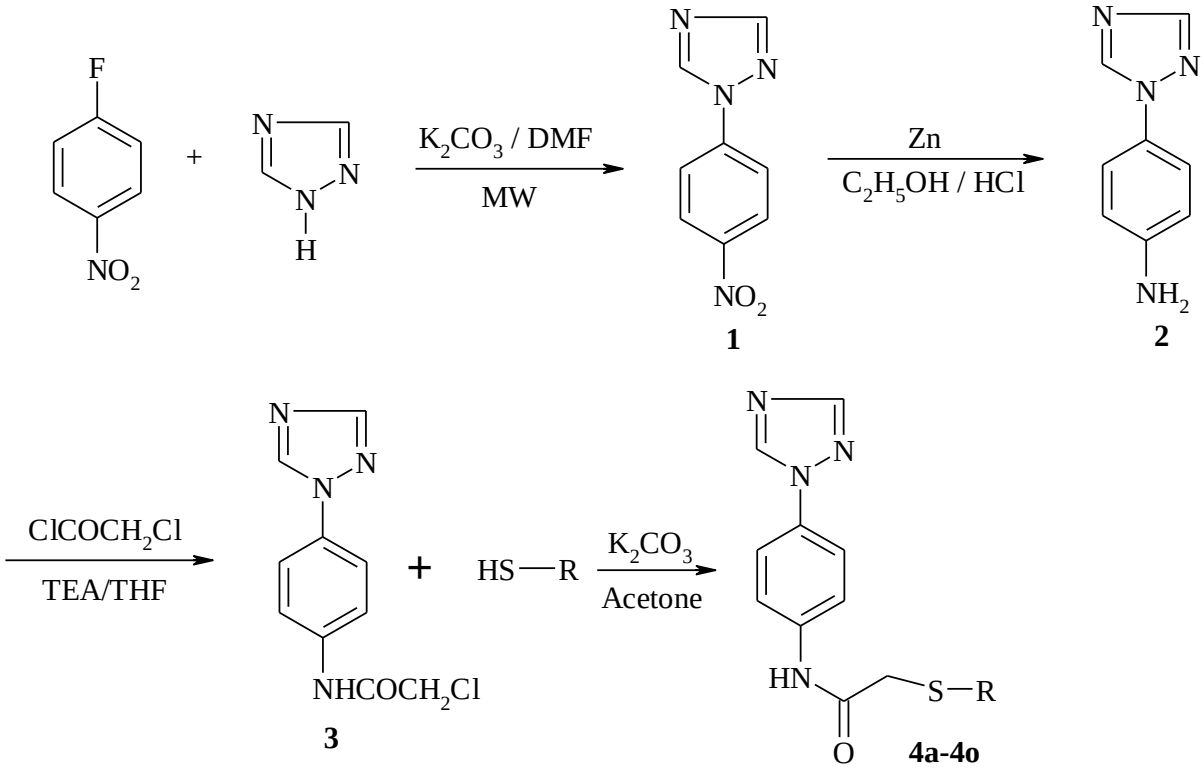
21. Wayne, PA,Reference method for broth dilution antifungal susceptibility testing of yeasts; approved standard-third edition; CLSI document M27-A3, Clinical and Laboratory Standards Institute, 2008.

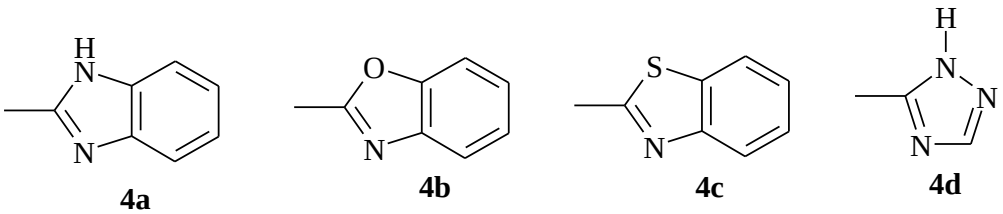
22. Ozkay Y, Tunalı Y, Karaca H, Işıkdag I, Antimicrobial activity of a new combination system of benzimidazole and various azoles,Arch Pharm 344, 264–271, 2011.

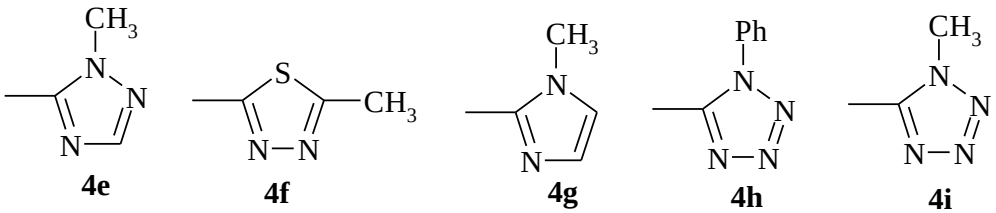
23. Ellman GL, Courtney KD, Andres V, Feather-Stone RM, A new and rapid colorimetric determination of acetylcholinesterase activity, BiochemPharmacol 7, 88-95, 1961.

24. Perry NSL, Houghton PJ, Theobald AE, Jenner P, Perry EK,In-vitro inhibition of human erythrocyte acetylcholine esterase by Salvia lavandulae folia essential oil and constituent terpenes,J Pharm Pharmacol 52, 895-902, 2000.

Scheme: Synthesis of 2-Substituted-sulfanyl-N-[4-(1,2,4-triazol-1-yl)phenyl]acetamide derivatives (**4a-4o**)







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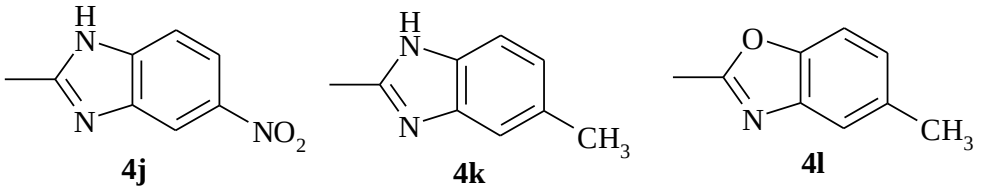

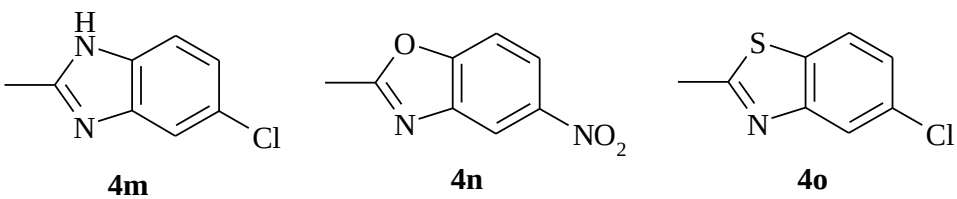

Table 1: MIC values (µg/mL) of the compounds (**4a-4o**) against *Candida* species.

Compound	*C. krusei(ATCC6258)*	*C. glabrata* (*ATCC90030)*	*C. albicans (ATCC1023)*	*C. parapsilosis (ATCC 7330)*
4a	200	100	200	400
4b	100	50	200	50
4c	400	200	200	100
4d	400	100	100	100
4e	200	100	100	100
4f	100	50	100	50
4g	200	50	100	100
4h	100	200	100	50
4i	100	100	200	200
4j	100	25	25	50
4k	100	25	25	100
4l	100	25	25	100
4m	100	100	400	400
4n	100	50	50	50
4o	50	400	200	200
Fluconazole	3.125	12.5	12.5	6.25

Table 2: % Inhibition potency of the compounds (**4a**-**4o**) at 1 and 0.1 mM concentrations.

Compound	**1**mM	**0.1**mM
4a	28.48±0.96	4.39±0.44
4b	27.63±1.87	15.92±1.21
4c	36.32±1.64	11.46±0.85
4d	18.03±0.42	7.40±0.27
4e	25.41±1.75	12.77±1.06
4f	39.21±3.16	25.17±2.41
4g	33.69±2.61	22.73±1.86
4h	18.46±2.09	9.68±0.81
4i	32.90±1.16	11.73±1.26
4j	20.91±0.98	8.63±0.69
4k	27.89±1.13	15.62±0.48
4l	17.43±1.15	11.40±1.63
4m	15.62±0.86	13.12±2.14
4n	16.27±0.79	8.85±1.30
4o	27.13±2.17	6.47±0.92
Donepezil	98.71±1.13	94.19±2.28