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RESEARCH ARTICLE

TETRAZOLE ANALOGUES: SYNTHESIS AND ANTIMICROBIAL ACTIVITY

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ABSTRACT

A series of new *N*1-(5-aryl/heteroaryl-1*H*-1,2,3,4-tetraazol-1-yl)-2-(2-morpholinoanilino)- acetamide 6 (a-i), has been synthesized by the cyclo-condensation of *N*'1-[(*E*)-1-aryl/heteroarylmethyl- dene]-2-(2-morpholinoanilino)ethanohydrazide (5a-i) witharyl/heteroaryl aldehyde. The structures of the synthesized compounds have been confirmed *via* IR, ^{1}H NMR, ^{13}C NMR and Mass spectral analyses. Further, all the newly synthesized compounds 6(a-i) have been assayed for their antimicrobial activity against Gram-positive and Gram-negative bacteria and fungi. The compounds containing moieties like 4-nitrophenyl (6c), 2-pyridyl (6g), 2-furyl (6h) and 2-thienyl (6i) exhibited good inhibitory activity against the tested organisms.

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INTRODUCTION

Tetrazoles are a class of heterocycles that have drawn interest because of their potential pharmacological activities, such as antihypertensive (Ramakrishna 2017), antimicrobial (Abdel 2013), corrosion inhibitor (Mohit 2010), anti-inflammatory (Sumar 2008), anticancer (Gorle 2017), antioxidant (Julliano 2017), analgesic (Shantaram 2013), antiviral (Hutchinson 1985), protein arginine deiminase inhibitor (Subramanian 2015), anti-allergic (Roger 1986), dual selective serotonin and nor epinephrine reuptake inhibitors (Paudel 2016)and HIV inhibitors (da Silva 2009). This nitrogen-rich ring structure is typically found in medications (Carini 1991, Koyama 1987, Raman 1980, Maxwell 1984), explosives (Saira2021), and propellants (Vereshchagin2024). Tetrazoles are also utilized as precursors of carbenes in flash vacuum pyrolysis (Bock 1987, Wentrup 1985, Wentrup 1984) and are significant synthons in synthetic organic chemistry (Burger 1991, Schelenz 2000). Numerous tetrazolebased compounds can form stable complexes with several metal ions (Frija 2009) and have demonstrated good coordination characteristics. Additionally, tetrazolyl halides have been effectively employed in organic synthesis as derivatizing agents for the chemical modification of alcohols due to the strong electron-withdrawing properties of the tetrazole ring (Arauo 2002, Araujo 2004, Frija 2005). Owing to the immense importance and varied bioactivities exhibited by tetrazole and its derivatives, in continuation of our ongoing research on the synthesis of new heterocyclic compounds, it was thought of interest to synthesize new heterocyclic compounds with potential biological activity. In this article, we wish to report the synthesis of new tetrazole analogues *N*1-(5-aryl/heteroaryl-1*H*-1,2,3,4-tetraazol-1-yl)-2-(2-morpholinoanilino)acetamide **6(a-i)** and evaluation of their *in vitro* antimicrobial activity.

MATARIALS AND METHODS

Commercial grade reagents were used as supplied. Solvents except analytical reagent grade were dried and purified according to the literature when necessary. Reaction progress and purity of the compounds were checked by thin-layer chromatography (TLC) on pre-coated silica gel F_{254} plates from Merck and compounds visualized either by exposure to UV light. Silica gel chromatographic columns (70–230 mesh) were used for separations. Melting points were determined on a Fisher–Johns apparatus and are uncorrected. IR spectra were recorded as KBr disks on a Perkin–Elmer FTIR spectrometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Gemini spectrometer (300 MHz for ^{1}H and 75 MHz for ^{13}C). Chemical shifts are reported as δ ppm against TMS as an internal standard and coupling constants (J) are reported in Hz units. Mass spectra were recorded on a VG micro mass 7070H spectrometer.

Synthesis of 2-morpholinoaniline (2): A solution of compound **1** (0.01 mol) in 20% hydrochloric acid (75 mL) was treated with small portion of zinc dust with stirring and gentle warming until all the orange colour of nitro compound had disappeared. The mixture was filtered to remove the excess zinc and the filtrate was neutralized with NaOH. The crude product obtained was filtered, dried and crystallized from methanol to give pure compound **2** in 84% yield. IR (KBr): 3370-3310, 3056, 1410, 1074 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 6.30-6.20 (m, 2H, ArH), 6.75-6.80 (m, 2H, ArH), 3.90-4.00 (bs, 2H, NH_2), 3.60-3.50 (m, 4H, CH_2O), 2.90-2.80 (m, 4H, CH_2-N).

Synthesis of ethyl 2-(2-morpholinoanilino)acetate (3): A mixture of compound **2** (0.01 mol) in 10 mL DMF and K_2CO_3 (0.01 mol) was stirred for 30 min and ethylchloroacetate (0.01 mol) was added

dropwise and the mixture was refluxed for 48 h, cooled the reaction mixture to precipitate the product. The crude product obtained was filtered and crystallized from ethylalcohol to give pure compound 3 in 62% yield. Yield 73%, IR (KBr) cm^{-1}: 3320, 3067, 1711, 1265, 1077; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.33 (t, 3H, CH_3), 3.40-3.50 (m, 4H, CH_2-N), 3.65-3.70 (m, 4H, CH_2-O), 3.83 (s, 2H, CH_2), 4.12 (q, 2H, CH_2), 6.30-6.50 (m, 4H, Ar-H), 6.92 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 15.4. 48.4, 49.2, 63.9, 65.7, 110.3, 114.2, 122.9, 123.4, 130.9, 139.7, 167.3; MS: *m/z* 265 (M^++1).

Synthesis of 2-(2-morpholinoanilino)ethanohydrazide (4): A mixture of compound **3** (0.01 mol) in 5 mL ethanol and excess of 80% hydrazine hydrate in ethanol (15 mL) was heated under reflux temperature for 8 hours. After completion of the reaction it was cooled and the solid separated was filtered and washed with water and then purified by recrystallization from ethanol to afford pure compound **4** in 61% of yield. IR (KBr) cm^{-1}: 3413, 3077, 1713, 1368; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.35-3.45 (m, 4H, CH_2-N), 3.65-3.70 (m, 4H, CH_2-O), 3.92 (s, 2H, CH_2), 5.72 (s, 2H, NH_2) 6.30-6.50 (m, 4H, Ar-H), 6.97 (s, 1H, NH); 8.11 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 47.4, 49.2, 65.9, 110.2, 114.8, 121.6, 124.5, 132.4, 139.7, 172.0; MS: *m/z* 251 (M^++1).

General procedure for the synthesis of *N*'1-[(*E*)-1-aryl/ heteroarylmethylidene]-2-(2-morpholinoanilino) ethanohydrazide (5a-i): To a stirred solution of ayl/heteroaryl aldehyde (0.01 mol) in absolute ethanol (10 mL) and 3-4 drops of glacial acetic acid was carefully added a solution of compound 4 (0.02 mol) in absolute ethanol (10 mL) and refluxed the reaction mixture with stirring for 8 h and then cooled, decanted the liquid to get crude product which was crystalized using ethyl alcohol to get corresponding pure compound **5(a-j)** in 54-71% of yields.

***N*'1-[(*E*)-1- phenylmethylidene]-2-(2-morpholinoanilino) ethanohydrazide (5a):** IR (KBr) cm^{-1}: 3433, 3071, 1705, 1467, 1078; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.35-3.45 (m, 4H, CH_2-N), 3.65-3.70 (m, 4H, CH_2-O), 3.98 (s, 2H, CH_2), 6.30-6.50 (m, 4H, Ar-H), 6.98 (s, 1H, NH); 7.15-7.20 (m, 5H, Ar-H), 7.92 (s, 1H, CH), 9.21 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 46.2, 49.2, 64.7, 110.7, 115.9, 121.3, 123.5, 126.7, 128.1, 129.0, 132.5, 133.4, 142.3, 145.6, 172.1, MS: *m/z* 338 (M^+).

General procedure for the synthesis of *N*1-(5-aryl/heteroaryl-1*H*-1,2,3,4-tetraazol-1-yl)-2-(2-morpholinoanilino)acetamide 6(a-i): A mixture ofcorresponding compound **5(a-i)** (0.01 mol) and PCl_5 (0.01 mol) was heated at 100 ^{0}C for one hour. When the evolution of fumes of HCl ceased, excess of PCl_5 was removed under reduced pressure and the residual imidoyl chloride was treated with an ice-cold solution of sodium azide (0.02 mol) and excess of sodium acetate in water (15 mL) and acetone (20 mL) with stirring. Stirring was continued for overnight, there after acetone was removed under reduced pressure. The remaining aqueous portion was extracted with chloroform was dried and recrystallized from ethanol to get the corresponding pure compounds **6(a-i)**.

***N*1-(5-phenyl-1*H*-1,2,3,4-tetraazol-1-yl)-2-(2-morpholinoanilino)acetamide (6a):** IR (KBr) cm^{-1}: 3412, 3062, 1707, 1259, 1251, 1097; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.40-3.45 (m, 4H, CH_2-N), 3.60-3.65 (m, 4H, CH_2-O), 4.12 (s, 2H, CH_2), 6.40-6.55 (m, 4H, Ar-H), 5.32 (s, 1H, NH); 5.82 (s, 1H, NH); 7.25-7.30 (m, 3H, Ar-H), 8.56 (d, *J* = 7.1 Hz, 2H, Ar-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 42.1, 49.8, 62.1, 113.4, 114.5, 121.0, 123.7, 127.2, 128.2, 130.7, 131.0, 133.7, 139.7, 154.7, 178.3; MS: *m/z* 380 (M^++1).

***N*1-[5 (4-chlorophenyl)1 *H*-1,2,3,4-tetraazol-1-yl]2-(2-morpholinoanilino) acetamide (6b):** IR (KBr) cm^{-1}: 3415, 3077, 1702, 1258, 1252, 1096, 686; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.40-3.45 (m, 4H, CH_2-N), 3.60-3.66 (m, 4H, CH_2-O), 4.14 (s, 2H, CH_2), 6.45-6.55 (m, 4H, Ar-H), 5.33 (s, 1H, NH); 5.84 (s, 1H, NH), 7.56 (d, *J* = 7.2 Hz, 2H, Ar-H), 8.92 (d, *J* = 7.2 Hz, 2H, Ar-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 42.4, 49.7, 62.3, 112.6, 114.1, 121.7, 123.4, 125.8, 127.5, 132.1, 133.5, 135.1, 139.5, 154.7, 177.4; MS: *m/z* 413 (M^+.

***N*1-[5- (4-nitrophenyl)-1*H*-1,2,3,4-tetraazol-1-yl]- 2-(2-morpholinoanilino)acetamide (6c):** IR (KBr) cm^{-1}: 3413, 3061, 1702, 1567, 1455, 1255, 1253, 1092; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.40-3.45 (m, 4H, CH_2-N), 3.60-3.65 (m, 4H, CH_2-O), 4.10 (s, 2H, CH_2), 6.40-6.50 (m, 4H, Ar-H), 5.34 (s, 1H, NH); 5.83 (s, 1H, NH), 8.15 (d, *J* = 7.8 Hz, 2H, Ar-H), 8.91 (d, *J* = 7.8 Hz, 2H, Ar-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 43.2, 49.2, 63.2, 113.0, 114.7, 121.1, 123.6, 124.0, 129.7, 133.4, 135.1, 139.5, 144.7, 155.1, 178.9; MS: *m/z* 425 (M^++1).

***N*1-[5-(4-methoxyphenyl)- 1*H*-1,2,3,4-tetraazol-1-yl]-2-(2-morpholinoanilino)acetamide (6d):** IR (KBr) cm^{-1}: 3398, 3061, 1702, 1256, 1252, 1091; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.40-3.45 (m, 4H, CH_2-N), 3.60-3.65 (m, 4H, CH_2-O), 3.80 (s, 3H, OCH_3), 4.12 (s, 2H, CH_2), 6.40-6.55 (m, 4H, Ar-H), 5.32 (s, 1H, NH); 5.84 (s, 1H, NH); 6.91 (d, *J* = 7.4 Hz, 2H, Ar-H), 8.96 (d, *J* = 7.4 Hz, 2H, Ar-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 42.4, 49.5, 58.9, 62.5, 113.7, 114.5, 115.1, 121.0, 122.8, 123.7, 128.7, 133.1, 139.5, 155.3, 160.6, 178.6; MS: *m/z* 410 (M^++1).

***N*1-[5- (2-hydroxyphenyl)- 1*H*-1,2,3,4 -tetraazol-1-yl]-2-(2-morpholinoanilino) acetamide (6e):** IR (KBr) cm^{-1}: 3407, 3064, 1703, 1256, 1251, 1093; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.40-3.50 (m, 4H, CH_2-N), 3.60-3.65 (m, 4H, CH_2-O), 4.09 (s, 2H, CH_2), 4.76 (s, 1H, OH), 6.40-6.55 (m, 4H, Ar-H), 5.31 (s, 1H, NH); 5.77 (s, 1H, NH); 6.80-7.00 (m, 3H, ArH) 8.41 (d, *J* = 7.6 Hz, 1H, Ar-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 42.4, 48.9, 62.4, 10.7, 113.1, 114.3, 118.1, 119.9, 121.3, 123.8, 128.9, 132.0, 133.1, 139.3, 159.7, 161.4, 179.1; MS: *m/z* 395 (M^+).

***N*1-5-[4-(dimethylamino) phenyl]-1*H*-1,2,3,4-t etraazol-1-yl-2(2-morpholinoanilino)aceta- mide (6f):** IR (KBr) cm^{-1}: 3417, 3060, 1705, 1256, 1254, 1092; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.91 (s, 6H, H_3), 3.40-3.45 (m, 4H, CH_2-N), 3.60-3.65 (m, 4H, CH_2-O), 4.12 (s, 2H, CH_2), 6.40-6.55 (m, 4H, Ar-H), 5.29 (s, 1H, NH); 5.77 (s, 1H, NH); 6.89 (d, *J* = 7.3 Hz, 2H, Ar-H), 8.87 (d, *J* = 7.3 Hz, 2H, Ar-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 41.2, 43.5, 49.1, 62.8, 110.7,113.9, 114.4, 122.1, 123.8, 124.5, 132.4, 133.5, 138.2, 148.7, 155.9, 178.8; MS: *m/z* 423 (M^++1).

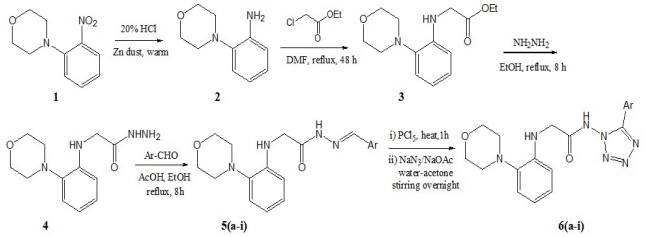
***N*1-[5- (2-pyridyl)-1 *H*-1,2,3,4-tetraazol-1-yl]-2-(2-morpholinoanilino) acetamide (6g):** IR (KBr) cm^{-1}: 3402, 3074, 1712, 1257, 1253, 1092; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.40-3.45 (m, 4H, CH_2-N), 3.60-3.65 (m, 4H, CH_2-O), 4.07 (s, 2H, CH_2), 6.40-6.55 (m, 4H, Ar-H), 5.35 (s, 1H, NH); 5.91 (s, 1H, NH), 7.19 (m, 1H, ArH), 8.10 (m, 1H, ArH), 8.80-8.90 (m, 2H, ArH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 43.2, 49.6, 62.3, 113.8, 114.5, 120.7, 122.2, 123.8, 124.2, 132.6, 135.6, 138.5, 147.6, 149.8, 153.7, 179.1; MS: *m/z* 381 (M^++1).

***N*1- [5-(2-furyl)- 1*H*-1,2,3,4-tetraazol-1-yl]-2-(2-morpholinoanilino) acetamide (6h):** IR (KBr) cm^{-1}: 3422, 3071, 1704, 1260, 1255, 1091; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.40-3.45 (m, 4H, CH_2-N), 3.60-3.65 (m, 4H, CH_2-O), 4.17 (s, 2H, CH_2), 6.40-6.55 (m, 4H, Ar-H), 5.34 (s, 1H, NH); 5.91 (s, 1H, NH), 6.59 (m, 1H, Ar-H), 7.20 (m, 1H, ArH), 8.12 (m, 1H, Ar-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 42.8, 49.6, 62.3, 105.6, 110.7, 113.7, 114.4, 121.9, 123.9, 133.5, 139.0, 141.2, 143.4, 145.1, 178.1; MS: *m/z* 370 (M^++1).

***N*1-[5- (2-thienyl)- 1*H*-1,2,3,4 -tetraazol-1-yl]-2-(2-morpholinoanilino) acetamide (6i):** IR (KBr) cm^{-1}: 3433, 3057, 1701, 1258, 1251, 1092; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.40-3.45 (m, 4H, CH_2-N), 3.60-3.65 (m, 4H, CH_2-O), 4.16 (s, 2H, CH_2), 6.40-6.55 (m, 4H, Ar-H), 5.35 (s, 1H, NH); 5.84 (s, 1H, NH), 6.33 (m, 1H, Ar-H), 7.78 (m, 1H, Ar-H), 8.44 (d, *J* = 7.8 Hz, 1H, ArH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 43.3, 49.3, 62.4, 113.6, 114.9, 121.4, 122.9, 123.8, 124.5, 128.7, 131.9, 133.6, 139.2, 142.7, 178.1; MS: *m/z* 386 (M^++1).

RESULTS AND DISCUSSION

The compound 1 on reduction with zinc dust in 20% HCl under stirring with gentle warming gave the 2-morpholinoaniline 2 in 84% yield. Compound 2 on reaction with ethylchloroacetate in presence of K_2CO_3 in DMF under reflux for 48 h, gave the ethyl 2-(2-morpholinoanilino)acetate 3 in 62% yield. The reaction of compound 3 with excess 80% hydrazine hydrate in ethanol, under reflux temperature for 8 h, afforded the 2-(2-morpholinoanilino) ethanohydrazide 4 in 61% yield. The condensation of 4 with corresponding aromatic/heteroaromatic aldehyde, in the presence of glacial acetic acid, in absolute ethanol at reflux for 8 h, afforded the corresponding *N*'1-[(*E*)-1-aryl/heteroaryl- methylidene]-2-(2-morpholinoanilino)ethanohydrazide5(a-i) in 54-71% yields. The corresponding compound 5(a-j) were treated with phosphorous pentachloride and heated at 100 ^{0}C for 1 h to get *in situ* imidoyl chloride intermediate which was treated with an ice-cold solution of sodium azide and excess of sodium acetate in water and acetone with stirring overnight, gave corresponding *N*1-(5-aryl/heteroaryl-1*H*-1,2,3,4-tetraazol-1-yl)-2-(2-morpholinoanilino)acet- amide 6 (a-i) in 48-62% of yields (Scheme 1). Chemical structures of all the newly prepared compounds were confirmed by their IR, ^{1}H NMR, ^{13}C NMR and MS spectral data. The IR spectrum of compound 6ashowed absorption bands in the region of 1251 (N=N), 1259 (C=N) of tetrazole ring, the carbonyl stretching in 1707 cm^{-1}. The additional support, for the formation of tetrazole ring, was obtained from Its ^{1}H NMR spectra which showed the signals at δ 3.40-3.45 and 3.60-3.65 ppm as multiplets, integrating four protons in each corresponding morpholine ring. Methylene and NH proton signals appeared at δ 4.12 as singlet, 5.32 and 5.82 as singlet respectively. All the other aromatic protons were observed at the expected regions. In the ^{13}C NMR spectrum, the C-5 carbon of tetrazole ring is observed at δ 154.7 ppm, the signals at δ 49.8 and 62.1 observed for morpholine ring, the carbonyl carbon appear at δ 178.3 ppm. The mass spectrum of compound showed $M^+ + 1$ peak at m/z: 380. In summary, all the newly synthesized compounds showed satisfactory spectral data consistent with their structures.



5/6: Ar= **a**) phenyl; **b**) 4-chlorophenyl; **c**) 4-nitrophenyl; **d**) 4-methoxyphenyl;**e**) 2-hydroxyphenyl; **f**) 4-N,N-dimethylaminophenyl; **g**) 2-pyridyl; **h**) 2-furyl; **i**) 2-thienyl

Scheme 1. Schematic route for the synthesis of compounds 6(a-i)

ANTIBACTERIAL ACTIVITY

All the newly synthesized compounds 6(a-i)were assayed for their antibacterial activity against Gram-positive bacteria *viz*. *Bacillus Subtilis* (MTCC 441), *Bacillus Sphaericus*(MTCC 11), and *Staphylococcus Aureus* (MTCC 96), and Gram-negative bacteria *viz*. *Pseudomonas Aeruginosa* (MTCC 741), *Klobsinella Aerogenes* (MTCC 39), and *ChromobacteriumViolaceum*(MTCC 2625) by disc diffusion and broth dilution methods (Villanova 1982). For the antibacterial assay, standard inoculums (1-2 × 10^{7}c.f.u/ml 0.5 Mc Farland standards) were introduced on to the surface of sterile agar plates, and a sterile glass spreader was used for even distribution of the inoculums. The discs measuring 6.26 mm in diameter were prepared from Whatman no.1 filter paper and sterilized by dry heat at 140 ^{o}C for 1 h. The sterile discs previously soaked in a known concentration of the test compounds were placed in nutrient agar medium. The plates were inverted and incubated for 24 h at 37 ^{o}C. The inhibition zones were measured and compared with the standard drug penicillin (Table 1). For the determination of MIC, bacteria were grown over night in Luria Bertani (LB) broth at 37 ^{o}C harvested by centrifugation, and then washed twice with sterile distilled water. Stock solutions of the series of compounds were prepared in DMSO. Each stock solution was diluted with standard method broth (Difco) to prepare serial two-fold dilutions in the range of 50–0.8 μg/ml. Ten microliters of the broth containing about 10^{5}c.f.u/ml of test bacteria were added to each well of 96–well microtiter plate. Culture plates were incubated for 24 h at 37 ^{o}C and the growth was monitored visually and spectrometrically. The minimal inhibitory concentrations (MIC, μg/ml) were measured and compared with the standard drug penicillin (Table 1). Antibacterial screening data revealed that all the tested compounds 6(a-i) are active and showed moderate to good antibacterial activity towards all the tested strains. Compounds containing 4-nitropheyl (6c) and 2-pyridyl (6g) moieties at 1-position and 2-position of the tetrazole ring respectively exhibited potent inhibitory activity towards all the tested microorganisms. Further, the compounds containing 2-furyl (6h)and 2-thienyl (6i) moieties showed good activity towards *B. subtilis* and *B. sphaericus*.

ANTIFUNGAL ACTIVITY

Compounds 6(a-i) were also evaluated for their antifungal activity against *Candida Albicans* (ATCC 10231), *Aspergillus Fumigatus* (HIC 6094), *Trichophyton rubrum* (IFO 9185) and *Trichophyton Mentagrophytes* (IFO 40996) in DMSO by disc diffusion, broth dilution methods (Villanova 1982). For the antifungal assay, Sabourands agar media was prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in distilled water (100 ml) and adjusting the *p*H to 5.7. Normal saline was used to make a suspension of spore of fungal strain for lining. A loopful of particular fungal strain was transferred to 3 ml saline to get a suspension of corresponding species. 20 ml of agar media was poured into each petri-dish, excess of suspension was decanted and the plates were dried by placing in an incubator at 37 ^{o}C for 1 h. Using an agar punch well were made and each well was labelled. A control was also prepared in triplicate and maintained at 37 ^{o}C for 3-4 days. The *C. albicans* was grown for 48 h at 28 ^{o}C in YPD broth (1% yeast extract, 2% peptone and 2% dextrose), harvested by centrifugation and then washed twice with sterile distilled water. *A. fumigatus*, *T. rubrum* and *T. mentagrophytes* were plated in potato dextrose agar (PDA) (Difco) and incubated at 28 °C for two weeks. Spores were washed three times with sterile distilled water and resuspended in distilled water to obtain an initial inoculums size of 10^5 spores/ml. Each test compound was dissolved in DMSO and diluted with potato dextrose broth (Difco) to prepare serial two-fold dilutions in the range 100 to 0.8 μg/ml.

Table 1: Antibacterial activity of compounds 6a-j

Compound	Minimal inhibitory concentration in μg/mL (zone of inhibition in mm)[a]					
	B. subtilis	*B. sphaericus*	*S. aureus*	*P. aeruginosa*	*K. aerogenes*	*C. violaceum*
6a	27 (10)	25 (12)	30 (10)	25 (9)	30 (5)	25 (8)
6b	40 (7)	35 (9)	30 (12)	35 (11)	25 (10)	28 (7)
6c	12 (20)	13 (20)	12 (29)	10 (19)	16 (21)	13 (21)
6d	22 (15)	20 (10)	25 (15)	19 (14)	27 (10)	20 (15)
6e	12 (18)	25 (16)	30 (12)	26 (10)	25 (10)	25 (11)
6f	25 (9)	30 (10)	28 (10)	28 (10)	30 (8)	28 (13)
6g	11 (22)	13 (18)	12 (26)	10 (19)	14 (15)	10 (18)
6h	15 (15)	13 (17)	30 (13)	40 (9)	25 (10)	30 (10)
6i	20 (17)	16 (19)	30 (11)	25 (10)	35 (9)	25 (8)
Penicillin	1.56 (25)	3.12 (28)	1.56 (40)	6.25 (25)	6.25 (30)	12.5 (25)

[a]The values in parentheses indicate the zone of inhibition.

Table 2. Antifungal activity of compounds 6a-j

Compound	Minimal inhibitory concentration in μg/mL (zone of inhibition in mm)[a]			
	C. albicans	*A. fumigatus*	*T. rubrum*	*T. mentagrophytes*
6a	30 (9)	35 (9)	35 (10)	30 (11)
6b	18 (20)	20 (17)	40 (8)	35 (15)
6c	25 (10)	15 (17)	35 (12)	25 (9)
6d	35 (11)	30 (10)	30 (10)	19 (19)
6e	25 (7)	30 (9)	35 (9)	30 (12)
6f	30 (6)	35 (12)	30 (10)	35 (12)
6g	27 (15)	20 (8)	25 (12)	16 (19)
6h	35 (13)	30 (10)	40 (14)	35 (13)
6i	15 (20)	20 (18)	25 (10)	20 (10)
Fluconazole	16 (22)	18 (20)	20 (22)	16 (20)

[a]The values in parentheses indicate the zone of inhibition.

Ten microliters of the broth containing about 10^3 (for yeast) and 10^4 (for filamentous fungi) cells/ml of test fungi was added to each well of a 96-well microtiter plate. Culture plates were incubated for about 48–72 h at 28°C. The inhibition zone and minimal inhibitory concentration (MIC) were determined and compared with the standard drug fluconazole (Table 2). The antifungal screening data reveal that, most of the newly synthesized compounds were active with moderate to good antifungal activity. The compounds containing 2-thienyl (6i)and 4-chlorophenyl (6b), moiety at 2-position and 1-position of the tetrazole ring respectively showed highest activity towards *C. albicans* and *A. fumigatus*. Further, the compound 6c containing 4-nitrophenyl moiety showed good antifungal activity towards *A. fumigatus*. The compounds 6d containing 4-methoxyphenyl and 6g containing 2-pyridyl ring also showed potent activity towards *T. mentagrophytes*.

CONCLUSION

A series of new *N*1-(5-aryl/heteroaryl-1*H*-1,2,3,4-tetraazol-1-yl)2-(2-morpholinoanilino)- acetamide **6 (a-i)**, has been synthesized and assayed for their antimicrobial activity against Gram-positive and Gram-negative bacteria and fungi. The compounds containing moieties like 4-nitrophenyl **(6c)**, 2-pyridyl **(6g)**, 2-furyl **(6h)** and 2-thienyl **(6i)** exhibited good inhibitory activity against the tested organisms.

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