GREEN CHEMISTRY APPROACH FOR THE SYNTHESIS OF NOVEL TETRAZOLE DERIVATIVES AND EVALUATION OF ANTIFUNGAL ACTIVITY

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New 2-substituted-4-(5-phenyl-1H-tetrazol-1-yl)-2,3,5a,9a-tetrahydro-1H-1,5-benzodiazepine derivatives were synthesized by conventional as well as microwave method. Benzonitrile and sodium azide in the presence of ammonium chloride and DMF produces 5-phenyltetrazole; this on reaction with acetic anhydride forms 5-phenyl-1-acetyl tetrazole which reacted with different aromatic aldehydes in the presence of the alkaline medium, to yield corresponding chalcones. Chalcones on further reaction with o-phenylenediamine yield 2-substituted-4-(5-phenyl-1H-tetrazol-1-yl)-2,3,5a,9a-tetrahydro-1H-1,5-benzodiazepines (**4a-4j**). The structures of newly synthesized compounds were characterized by physical and spectral characteristics by FT-IR and ^{1}H NMR spectroscopy. All synthesized compounds were evaluated for their antifungal activity by MIC (minimal inhibitory concentration, broth dilution method) against *A. niger* and C. *albicans*. All synthesized compounds show moderate to good antifungal activity.

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Introduction

Tetrazoles have been attracted as an important class of heterocyclic compounds in the field of clinical research and medicinal chemistry. Tetrazoles have not been found in nature, but they are resistant to biological degradation. This property makes it possible to use tetrazoles as isosteric substituents of various functional groups in the development of biologically active substances.[1] Tetrazole and their derivatives have great importance in pharmaceutical chemistry due to their diverse biological activity such as antifungal,[2] antibacterial,[3] antiinflammatory,[4] antituberculous,[5] antihypertensive agents,[6] anticancer,[7] antibiotic[8] and anticonvulsant.[9]

Development of the tetrazole chemistry has mainly been associated with the wide-scale application of these compounds in medicine, biochemistry and agriculture. The tetrazole functionality plays a vital role in medicinal chemistry, primarily due to its ability to serve as the bioequivalent (bioisoster) of the carboxylic acid group. In particular, 1-substituted tetrazoles and 5-thio-substituted tetrazoles have been used in the synthesis of pharmacologically active drugs.[3]

The 1,5–benzodiazepines moiety is a privileged class of pharmacophore, as compounds bearing this structural unit possess a broad spectrum of biological activities, as antimicrobial,[10] anti-inflammatory[11], anticancer[12] and anticonvulsant activities.[13] The synthesis of the 1,5–benzodiazepines moiety involves the reaction of chalcones with o-phenylenediamine.[14] Tetrazoles clubbed with benzodiazepines will help to improve the antifungal properties of the pharmacophore leading to more potent compounds.

In recent years, organic reactions involving a green chemistry approach have received considerable attention in organic synthesis because of their ease handling, enhanced reaction rates, more excellent selectivity, simple workup and recoverability of the products.[15] The synthesis of novel tetrazole based benzodiazepines derivatives and investigation of their chemical and biological behavior has gained more importance in recent decades for biological and pharmaceutical reasons.

In continuation of research in the field of green chemistry, an attempt is made to synthesize tetrazole containing benzodiazepine and the compounds have been evaluated for antifungal activity, which has not been reported yet.

Experimental

Melting points were determined with open capillary and were uncorrected. FT-IR spectra were recorded on a 'JASCO FT-IR-4600' spectrophotometer, ^{1}H-NMR spectra were recorded in BRUKER AVANCE II400'NMR spectrometer at 400 MHz frequency in DMSO using TMS as an internal standard.

Synthesis of 5-phenyltetrazole (1)

A mixture of benzonitrile (3.3 g, 0.10 mol), sodium azide (0.65 g, 0.10 mol) dimethylformamide (10 mL) and ammonium chloride (5.3 g, 0.10 mol) was heated in an oil bath for 7 h at 125 °C. The solvent was removed under reduced pressure. The residue was dissolved in 100 mL of

water and carefully acidified with concentrated hydrochloric acid to pH 2. The solution was cooled to 5 °C in an ice bath. Compound **1** has been recrystallized from aqueous methanol.

Synthesis of 5-phenyl-1-acetyltetrazole (2)

A solution of 5-phenyl tetrazole (12.8 g, 0.08 mol), acetic anhydride (0.08 mol) and 2-3 drops of concentrated sulphuric acid were heated for 15-20 min on a water bath, then cooled and poured into ice-cold water. The product was filtered and dried and recrystallized from ethanol.

General procedure for the preparation of chalcones (3a-3j): Method 1. Conventional synthesis

A solution of 5-phenyl-1-acetyltetrazole (8.5g, 0.005 mol) and the aromatic aldehyde (0.005 mol) in ethanol (12 mL) was cooled to 5 to 10 °C in an ice bath. The cooled solution was treated with dropwise addition of aqueous potassium hydroxide (2.5 mL, 50 %). The reaction mixture was stirred for 30 min and then left overnight. The resulting dark solution was diluted with ice water and carefully acidified using diluted hydrochloric acid. The chalcone was collected by filtration and washed with aqueous sodium bicarbonate and water then recrystallized from ethanol.

Method 2. Microwave-assisted synthesis

A mixture of 0.01 mol 5-phenyl-1-acetyltetrazoles, 0.01 mol of aromatic aldehydes, ethanol (5 mL) and 2.5 mL of NaOH (6 M) was kept in a microwave oven at level 2 and time for 2 min. The removed mixture was cooled in an ice-bath and acidified with concd. HCl. The chalcone was collected by filtration.

Synthesis of substituted tetrazoles derivatives (4a-4j) - Method 1. Conventional synthesis

A mixture of chalcone (**3a-h**) (0.01 mol) and o-phenylenediamine (0.01 mol) was dissolved in absolute ethanol (30 mL) in the presence of 20 % aq. NaOH, and the reaction mixture was refluxed for about 5 h. After completion of the reaction, the reaction mixture was poured into crushed ice. The product obtained was filtered, washed with cold water. The compounds were obtained as yellow, brown, or dark brown crystals and they were recrystallized from ethanol

Method 2. Microwave-assisted synthesis

A mixture of 0.1 mol of chalcone (**3a-j**), 0.1 mol of o-phenylenediamine, ethanol (5 mL) and 3 ml of NaOH solution (6 M) was kept in a microwave oven at level 3 and time for 3 min. The removed mixture was cooled in an ice-bath and acidified with concd. HCl. The synthesized tetrazole derivatives were collected by filtration.

Spectral data of compounds: [IR (KBr), ν, cm^{-1} and ^{1}H-NMR (DMSO), δ ppm]

2-(4-Chlorophenyl)-4-(5-phenyl-1H-tetrazol-1-yl)-2,3,5a,9a-tetrahydro-1H-1,5-benzodiazepine (4a)

FT-IR: 1228 (N-N=N-), 1140 (tetrazole), 3565 (N-H), 1652 (C=C), 2929 (Ar-CH), 1521 (C=N). ^{1}H-NMR: 3.2-5.3 (5H, m, benzodiazepine), 6.5-7.9 (13H, m, Ar-H).

4-[4-(5-Phenyl-1H-tetrazol-1-yl)-2,3,5a,9a-tetrahydro-1H-1,5-benzodiazepin-2-yl]phenol (4b)

FT-IR: 1338 (N-N=N-), 1108 (tetrazole), 3586 (N-H), 1646 (C=C), 2969 (Ar-CH), 1507 (C=N). ^{1}H-NMR: 3.1-5.2 (5H, m, benzodiazepine), 5.4(1H, s, OH), 6.6-7.8 (13H, m, Ar-H).

2-(4-Bromophenyl)-4-(5-phenyl-1H-tetrazol-1-yl)-2,3,5a,9a-tetrahydro-1H-1,5-benzodiazepine (4c)

FT-IR:1216 (N-N=N-), 1116 (tetrazole), 3637 (N-H), 1638 (C=C), 2969 (Ar-CH), 1589 (C=N). ^{1}H-NMR: 3.2-5.3 (5H, m, benzodiazepine), 6.6-7.8(13H, m, Ar-H).

2-(2,4-Dimethoxy)-4-(5-phenyl-1H-tetrazol-1-yl)-2,3,5a,9a-tetrahydro-1H-1,5-benzodiazepine (4d)

FT-IR:1216 (N-N=N-), 1123 (tetrazole), 3637 (N-H), 1635 (C=C), 2956 (Ar-CH), 1540 (C=N). ^{1}H-NMR: 3.2-5.3 (5H, m, benzodiazepine), 6.6-7.8 (12H, m, Ar-H), 2.27 (6H, s, OCH_3)

2-(4-Nitrophenyl)-4-(5-phenyl-1H-tetrazol-1-yl)-2,3,5a,9a-tetrahydro-1H-1,5-benzodiazepine (4e)

FT-IR:1216 (N-N=N-), 1092 (tetrazole), 3446 (N-H), 1683 (C=C), 2959 (Ar-CH), 1558 (C=N). ^{1}H-NMR: 3.2-5.4(5H, m, benzodiazepine),6.4-7.9(13H, m, Ar-H).

2-(2-Chlorophenyl)-4-(5-phenyl-1H-tetrazol-1-yl)-2,3,5a,9a-tetrahydro-1H-1,5-benzodiazepine (4f)

FT-IR: 1228 (N-N=N-), 1116 (tetrazole), 3565 (N-H), 1652 (C=C), 2929 (Ar-CH), 1521 (C=N). ^{1}H-NMR: 3.2-5.3 (5H, m, benzodiazepine), 6.5-7.9 (13H, m, Ar-H).

2-(3-Nitrophenyl)-4-(5-phenyl-1H-tetrazol-1-yl)-2,3,5a,9a-tetrahydro-1H-1,5-benzodiazepine (4g)

FT-IR:1216 (N-N=N-), 1183 (tetrazole), 3446 (N-H), 1683 (C=C), 2959 (Ar-CH), 1558 (C=N). ^{1}H-NMR: 3.4-5.3(5H, m, benzodiazepine), 6.3-7.9(13H, m, Ar-H).

2-(Furoyl-2-yl)-4-(5-phenyl-1H-tetrazol-1-yl)-2,3,5a,9a-tetrahydro-1H-1,5-benzodiazepine (4h)

FT-IR: 1215 (N-N=N-), 1120(tetrazole), 3524 (N-H), 1636 (C=C), 3013 (Ar-CH), 1540 (C=N). ^{1}H-NMR: 3.2-5.3 (5H, m, benzodiazepine), 6.5-7.9 (12H, m, Ar-H).

2-(4-Dimethylamino)-4-(5-phenyl-1H-tetrazol-1-yl)-2,3,5a,9a-tetrahydro-1H-1,5-benzodiazepine (4i)

FT-IR: 1218 (N-N=N-), 1133 (tetrazole), 3565 (N-H), 1652 (C=C), 2968 (Ar-CH), 1540 (C=N). ^{1}H-NMR: 3.2-5.3 (5H, m, benzodiazepine), 6.5-7.9 (13H, m, Ar-H), 2.7 (6H, s, $(CH3)_2$)

2-(4-Dimethylamino)-4-(5-phenyl-1H-tetrazol-1-yl)-2,3,5a,9a-tetrahydro-1H-1,5-benzodiazepine.

FT-IR: 1224 (N-N=N-), 1119 (tetrazole), 3545 (N-H), 1652 (C=C), 2924 (Ar-CH), 1507 (C=N). ^{1}H-NMR: 3.2-5.6 (5H, m, benzodiazepine), 6.7-8.1(14H, m, Ar-H), 6.3-6.5 (2H, s, CH=CH) **(4j)**

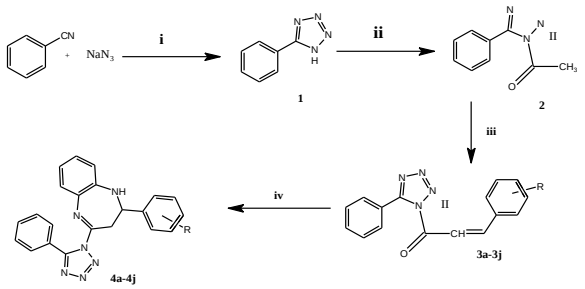
Antifungal activity-

The synthesized compounds were screened for antifungal activity by using MIC (minimal inhibitory concentration, broth dilution method). A Sabouraud-dextrose media (double strength test tubes) was prepared. A test tube without inoculum was used as a negative control. Inoculums (three to four drops) are added to reach the requested concentration of microorganism (10^6 cell/test tubes); The test compounds were added ranging from 0.5 to 5 mL except for uninoculated (negative control) and control (positive control) tube. The final volume was adjusted (10 mL) by using sterile water. All test tubes are properly shaken and then incubated at 37 °C for two days.

Results and discussions

The tetrazole derivatives were synthesized using conventional as well as microwave-assisted synthesis methods according to Scheme. Spectral data confirmed the structure of all synthesized derivative.

5-Phenyltetrazole (compound **1)** was prepared by the reaction of benzonitrile with sodium azide in the presence of ammonium chloride and DMF. 5-Phenyltetrazole (**1**) was converted to 5-phenyl-1-acetyltetrazole (**2**) by the reaction with acetic anhydride and sulphuric acid. Compounds **3a-3j** were obtained by treatment of **2** with aromatic aldehydes in the presence of NaOH. Compounds **3a-3j** on treatment with o-phenylenediamine in the presence of ethanol and NaOH yielded a compounds **4a-4j**, respectively.



Reagents and condition: i) DMF/ammonium chloride (conventional method), ii) acetic anhydride/H_2SO_4 (conventional method); iii) ArCHO/NaOH (conventional or microwave method); iv) o-Phenylenediamine (OPD)/NaOH (conventional or microwave method)

The IR spectra of compounds **4a-4j** show absorption bands at 2929 cm^{-1} due to Ar-H and at 1625 cm^{-1} due to C=N ring stretches. Absorption bands occur at 1280 (N-N=N-), 1108 and 1140 cm^{-1} (tetrazole ring).

The ^{1}H-NMR spectra show the chemical shift at 6.9-7.8 due to aromatic protons, 3.2-5.6 due to (5H benzodiazepine part). The results of spectral data are in good agreement with the structure of synthesized compounds.

Table 1. Physicochemical data of compounds **4a-4j** prepared by conventional (CM) and microwave-assisted (MW) methods

	'R' group	Molecular formula	M.wt.	Time		M.P. (^{0}C)		% yield		R_f Value
				CM, h	MW, min	CM	MW	CM	MW	
4a	4-Cl	$C_{22}H_{19}ClN_6$	402	5	3	187	186	65.6	70.6	0.56
4b	4-OH	$C_{22}H_{20}N_6O$	384	5	3	155	155	62.5	68.9	0.63
4c	4-Br	$C_{22}H_{19}BrN_6$	447	5	3	160	161	66.5	74.9	0.69
4d	2,4-$(OMe)_2$	$C_{24}H_{24}N_6O_2$	428	5	3	190	188	56.4	65.8	0.67
4e	4-NO_2	$C_{22}H_{19}N_7O_2$	413	5	3	150	149	74.3	80.9	0.52
4f	2-Cl	$C_{22}H_{19}ClN_6$	402	5	3	181	182	72.5	75.9	0.66
4g	3-NO_2	$C_{22}H_{19}N_7O_2$	413	5	3	154	154	59.8	70.8	0.45
4h	Furoyl	$C_{20}H_{18}N_6O$	358	5	3	145	146	68.3	70.8	0.62
4i	4-NMe_2	$C_{24}H_{25}N_7$	411	5	3	165	164	71.1	68.5	0.55
4j	cinnamoyl	$C_{24}H_{22}N_6$	394	5	3	172	172	63.5	66.8	0.56

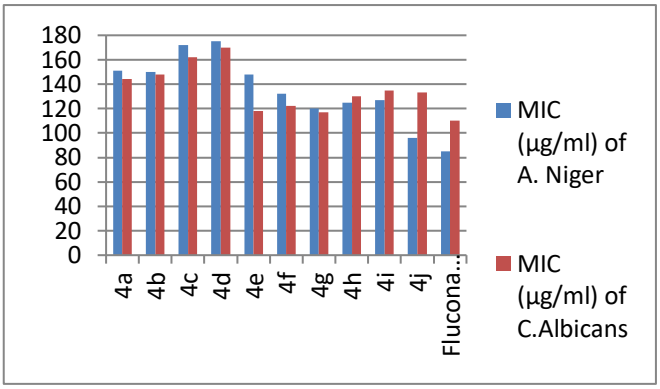


Figure 1. Antifungal activities of synthesized compounds (**4a-4j**)

The results of antifungal activity are depicted in Table 2 and Fig. 1, revealing that all compounds show antifungal activity against *Aspergillus niger* and *Candida albicans.* The activities are comparable with control standard Fluconazole shows potent activity at MIC of 85 and 110 µg mL^{-1}.

Table 2. Anti-fungal activity of synthesized compounds (**4a-4j**)

Compound	MIC, µg mL^{-1}	
	A. niger	***C. Albicans***
4a	148	144
4b	125	148
4c	172	117
4d	175	170
4e	148	118
4f	132	122
4g	120	142
4h	172	161
4i	150	146
4j	172	166
Fluconazole	85	110

Compounds **4b** and **4g** (4-OH, 3-NO_2) have shown good antifungal activity against *A. niger* while compounds, **4c, 4e** and **4f**, (4-Br, 4-NO_2, 2-Cl) have shown good antifungal activity against *C. Albicans.* Compounds **4a** and **4i** (4-Cl, 4-$N(CH_3)_2$) have shown moderate while compounds **4d, 4h** and **4j** showed weak antifungal activity against *A. niger* and *C. albicans.*

Conclusions

Tetrazole derivatives were synthesized from 5-phenyl-tetrazole which was synthesized from benzonitrile and sodium azide in good yields. The compounds **4b** and **4g** possess 4-OH, 3-NO_2 potent anti-inflammatory activity in comparison with control. The compounds **4c, 4e** and **4f** containing 4-Br, 4-NO_2, 2-Cl substitution produce moderate anti-inflammatory activity. The compounds **4d, 4h** and **4j** have shown weak anti-fungal activity against *A. niger* and *C. Albicans.*

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