SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF TETRAZOLE DERIVATIVES

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ABSTRACT

Tetrazoles are an important class of heterocyclic compounds which possess wide spectrum of biological properties. A series of tetrazoles were synthesized by diazotization of aminoguanidine bicarbonate using nitric acid and sodium nitrate to yield guanylazide. The obtained diazotisation mixture was refluxed using sodium carbonate to obtain 5-aminotetrazole which was acetylated using acetyl chloride and condensed using substituted aromatic aldehydes. All the synthesized compounds were characterized by IR, 1H NMR and 13C NMR Spectroscopy. The compounds were evaluated for their antibacterial activity against Staphylococcus aureus Bacillus pimilis and Escherichia coli using streptomycin as reference standard and anti-fungal activity against Aspergillus niger and Pencillium notatum using miconazole as reference standard. Compounds TZ2 and TZ8 were found to exhibit highest activity against S.aureus and B.pimilis. The compounds TZ2 and TZ7 showed maximum activity against E. coli. The compounds TZ1 and TZ7 exhibited most potent activity against A. niger and P. notatum respectively.


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INTRODUCTION

Tetrazoles are a class of synthetic organic heterocyclic compound (Fig. 1), consisting of a 5-member ring of four nitrogen and one carbon atom [1]. The majority of tetrazoles are crystalline solids. In general most of the tetrazoles are acids and often yield explosive salts. Tetrazoles are generally soluble in polar solvents and insoluble in non polar solvents, 1H-tetrazoles has good solubility in water. Unsubstituted tetrazole and C-substituted 1-H tetrazoles show amphoteric properties, they are weak NH-acids and readily form salts with strong mineral acids [2-4]. Tetrazole and its derivatives enter electrophilic and nucleophilic substitution reactions generally on the 5 ring position. Thermal destruction of tetrazole cycle usually takes place at 150-200°C [5, 6].

From the thorough literature survey it was found that tetrazole derivatives possess very interesting pharmacological and biological properties and are reported to exhibit variety of biological activities like antibacterial, antifungal and anticonvulsant, analgesic, anti-inflammatory, anti-tubercular activity and anticancer activity. Similarly 1, 5 disubstituted tetrazoles have long been known for their pharmaceutical activity as stimulants or depressants on the central nervous system and are reported to show oral antidiabetic and antithrombotic and antimicrobial properties [7, 8].

![Fig. 1 Tetrazole.](image)

As a part of our continuing interest in the area of antimicrobial derivatives, we began a study of antibacterial and antifungal screening of novel tetrazole derivatives. Although tetrazoles have been known from long ago to be biologically active, their varied biological features are still of great scientific interest. Since the existing drugs are developing resistance against bacterial strains we have planned for the synthesis of some new tetrazole derivatives with a hope to get better molecules. As part of our research in the development of synthetic routes to a new group of biologically active heterocyclic compounds, a series of compounds containing tetrazole ring have been synthesized and evaluated for their antimicrobial activity.

EXPERIMENTAL WORK:

Materials required:

Aminoguanidine bicarbonate, nitric acid, sodium nitrite, sodium carbonate, sulphuric acid, 5-amino tetrazole, acetyl chloride, different benzaldehydes, different ketones, potassium hydroxide, zinc chloride, thioglycolic acid, O-hydroxyacetophenone, substituted ethyl acetoacetates, glacial acetic acid, P-dimethyl benzaldehydes, sodium hydroxide, DMF, POCl3, NaHCO3, pet.ether, chloroform, ethanol. All these chemicals were bought from National Scientific Products, Sambasivapet, Guntur.

Instruments for characterization:

IR instrument used was Spercle Elmer DHF-1 FT-IR for IR spectra, Bruker AMX400 MHz NMR for 1H and C13 NMR and for mass spectroscopy Agilent 1100 Series LC-MSD at Liala implex, Vijayawada.

SCHEME 1:

![Scheme 1](image)

Fig. 2 Synthesis of (E)-N-(1H-tetrazol-5-yl)-substituted amide.
SYNTHESIS

Synthesis of 5-Amino tetrazole (1): Thiele method: 34g (0.25mol) of amino guanidine bicarbonate is added to 217ml of 15% nitric acid (0.561mol), and mixed until evaluation of carbon dioxide is stopped and resulted amino guanidine nitrate is fully dissolved in solution. Yellow transparent solution is diazotised by slow addition of 17.2g sodium nitrite (0.25mol) in 35ml of water. Addition is accompanied by stirring, and temperature during all addition period is kept between 20-25°C by using water bath if needed. After completion of reaction the diazotisation mixture is allowed to sit for 20 min at room temperature. And 29g of sodium carbonate is added (or 46g of sodium bicarbonate). Mixture is then heated on a water bath and refluxed for 4hrs. The solution is then neutralized by 30% sulphuric acid to pH 4, cooled to room temperature and allowed to sit over night. The precipitated crystals of 5-amino tetrazole monohydrate are filtered, washed with cold water and dried. Yield is about 70-74% based on amino guanidine [9-11].

Synthesis of 5-acetyl tetrazole (2): To a suspension of 5-amino tetrazole (0.04mol) in water (12ml) in a conical flask is added acetyl chloride (0.04mol). The mixture is stirred vigorously and warmed on water bath. The solid dissolves. After 10 minutes the solution is cooled and the separated acetyl tetrazole is filtered and washed with cold water. It is recrystallised from hot water [12].

Synthesis of (E)-N-(1H-tetrazol-5-yl)-substituted amide (3): A solution of 5-acetyl tetrazole (0.010mol) and aromatic aldehydes (0.010mol) in ethanol was cooled to 5-10°C in an ice bath. (For different substitutions refer Table 1). The cooled solution was treated with drop wise addition of aqueous potassium hydroxide (2.5 ml, 50%). The reaction mixture was magnetically stirred for 30min and then left over night. The resulting dark solution was diluted with ice cold water and carefully acidified using diluted hydrochloric acid. Compounds TZ1, TZ2 and TZ3 were obtained as crystalline mass. The obtained tetrazole analogues of chalcone were collected by filtration by washing with sodium bicarbonate and water. It was further purified by crystallization from ethanol [13, 14]. For scheme refer Fig 2.

Physical and Spectral data

TZ-1: Pale yellow solid, soluble in chloroform, IR (KBr): Cm⁻¹ = 1585.86 (N-H; Str), 1280.71(C=N; Str), 1676 (C=O; Str), 1676(C=C; Str), 681.54(C-Cl; Str); ñHNMR: δppm= 7.46 (d, 1H, COCH=), 7.2 (s, 1H, Ar-CH), 8.05 (d, 1H, CH), 8.0 (s, 1H, NH); C¹³: δppm= 75.98 (Ar-C=), 77.39 (Ar-C=), 77.81 (Ar-C=), 126.45 (Ar-C=), 129.93 (Ar-C=), 131.06 (Ar-C=), 143.79 (-C=), 173.15 (C=O)

TZ-2: Yellow solid, soluble in chloroform, IR (KBr): Cm⁻¹ = 1603.10 (N=; Str), 1569.38(N-H; Bend), 1664.52(C=O; Str), 1603.10,1569.38(C=C; Str); ñHNMR: δppm= 1.8 (s, 3H, CH₃), 2.58(d, 1H, COCH=), 7.28 (m, 3H, Ar- CH), 8.02 (d, 1H, CH); C¹³: δppm= 29.69 (CH₃), 76.68 (Ar-C=), 76.99 (Ar-C=), 77.31 (Ar-C=), 126.69 (Ar-C=), 129.19 (Ar-C=), 130.26 (Ar-C=), 144.59 (-C=), 172.29 (C=O).

TZ-3: Pale yellow solid, soluble in chloroform, IR (KBr): Cm⁻¹ = 1603.45 (N=; Str), 1569.12(N-H; Bend), 1664.39(C=O; Str), 1603.45, 1569.12(C=C; Str); ñHNMR: δppm= 2.85 (s, 6H, NH), 6.54(d, 2H, Ar- CH), 6.84 (s, 1H, COCH=), 7.12(d, 2H, Ar- CH), 7.55 (s, 1H, CH); C¹³: δppm= 31.95 (CH₃), 76.68 (Ar-C=), 76.56 (Ar-C=), 77.32 (Ar-C=), 125.88 (Ar-C=), 129.96 (Ar-C=), 130.76 (Ar-C=), 144.25 (-C=), 173.06 (C=O).

Table 1: Different substitutions of compound 3 in scheme 1.

<table>
<thead>
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<th>S. No.</th>
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<td>CH₃</td>
</tr>
<tr>
<td>3</td>
<td>TZ-3</td>
<td>HN&lt;CH₃&gt;</td>
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</table>
**Scheme 2:**

Aminoguanidine Bicarbonate $\rightarrow$ Guanylazide $\rightarrow$ Reflux $\rightarrow$ Ethanol

**Fig. 3** Synthesis of 2, 3-dihydro-2-substituted phenyl-3-(1H tetrazol-5-yl)thiazol-4-one.

**Table 2:** Different substitutions of compound 3 in scheme 2.

<table>
<thead>
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<th>S. No.</th>
<th>Code</th>
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</tr>
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<tbody>
<tr>
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<td>TZ-4</td>
<td>![Structure]</td>
</tr>
<tr>
<td>2</td>
<td>TZ-5</td>
<td>![Structure]</td>
</tr>
<tr>
<td>3</td>
<td>TZ-6</td>
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<td>TZ-7</td>
<td>![Structure]</td>
</tr>
<tr>
<td>5</td>
<td>TZ-8</td>
<td>![Structure]</td>
</tr>
</tbody>
</table>

**Synthesis**


Synthesis of N-Substituted benzylidene-1H-tetrazol-5-amine (2): To the mixture of (0.01mol) of compound was added (0.01mol) substituted benzaldehydes (For different substitutions refer Table 2) and 20ml of ethanol taken in RBF and was refluxed on a water bath for 2hrs. The resultant solution was cooled; the solid that separated was filtered and recrystallized from pet.ether.
Synthesis of 2,3-dihydro-2-substituted phenyl-3-(1H tetrazoll-5-yl)thiazol-4-one (3): To mixture of compound, 8ml of ethanol (0.2mol), 0.1g of zinc chloride and 0.4ml (0.004mol) of thioglycolic acid was added and the mixture was refluxed for 8hrs. After that the solid was separated out. Compounds TZ4 to TZ8 were obtained. For scheme refer Fig. 3.

Physical and Spectral data
TZ-4: Pale yellow solid, soluble in ethanol, IR (KBr): Cm\(^{-1}\) = 1648.36(N=N; Str), 3356.99(N-H; Str), 1044.79(C-N; Str), 1153.97(C=S; Str), 1648.38(C=O; Str), 747.19(C-Br; Str); \(^1\)HNMR: \(\delta\)ppm = 4.9 (s, 1H, CH), 6.95(d, 2H, Ar-CH), 7.31(d, 2H, Ar-CH).

TZ-5: Pale yellow solid, soluble in ethanol, IR (KBr): Cm\(^{-1}\) = 1648.05(N=N;Str), 3357.41(N-H; Str), 1284.52(C-N; Str), 1648.05(C=N;Str), 663.70(C=S; Str), 747.74(C-Cl;Str), 1648.05(C=O;Str); \(^1\)HNMR: \(\delta\)ppm = 4.9 (s, 1H, CH), 7.0(d, 2H, Ar-CH), 7.15(d, 2H, Ar-CH); Mass: m/z 296(M).

TZ-6: White solid, soluble in chloroform, IR (KBr): Cm\(^{-1}\) = 1556.31(N=N;Str), 3356.22(N-H; Str), 1046.30(C-N; Str), 1079.43(C=S; Str), 1647.73(C=O, Str), 747.69(C-Br, Bend), 1647.73(C=N; Str); \(^1\)HNMR: \(\delta\)ppm = 2.35 (s, 1H, CH3), 4.9(d, 1H, CH), 7.15(d, 4H, Ar-CH).

TZ-7: Pale yellow solid, soluble in chloroform, IR (KBr): Cm\(^{-1}\) = 3357.8(N-H; Str), 1081.00(C-N; Str), 1649.35(C=S; Str), 1046.11(C=O; Str), 1556.69(C=C, Str), 1752.49(C=O, Str), 1155.30(C=S; Str); \(^1\)HNMR: \(\delta\)ppm = 4.9 (s, 1H, CH), 6.85(d, 2H,Ar-CH), 7.04(d, 4H, Ar-CH).

TZ-8: White solid, soluble in methanol, IR (KBr): Cm\(^{-1}\)=3352.72(N=H; Str), 1047.68(C-N; Str), 1642.00(C=N; Str), 1758.68(C=O; Str), 1154.63(C=S; Str), 663.33(C-S; Str); \(^1\)HNMR: \(\delta\)ppm = 2.50(s, 1H, CH), 3.32 (s, 1H, CH), 6.42 (s, 1H, CH), 14.32 (s, 1H, NH)

BIOLOGICAL EVALUATION

Antibacterial activity:
Method: Cup plate method was used to carry out this study.
A. Preparation of test solutions:
Synthesized compound of Quinolinyl Pyrazolines were dissolved in minimum amount of Dimethyl Sulfoxide (DMSO) and the final volume made with DMSO, to get 50 and 100 μg/ml concentrations.

B. Preparation of standard solutions:
Streptomycin was the reference standard drug prepared in DMSO to get 50 and 100 μg/ml concentrations.

B. Preparation of standard solutions:
Streptomycin was the reference standard drug prepared in DMSO to get 50 μg/ml.

C. Test organisms used were:

1. Escherichia coli. (NCIM 2607) -Gram -ve
2. Staphylococcus aureus -Gram +ve
3. Bacillus pimilis.(NCIM 2063)

D. Preparation of sub culturing media:
Peptone water media was prepared using following ingredients.
1. Beef extract – 10 g
2. Peptone – 10 g
3. Sodium chloride – 5 g
4. Distilled water – Q.S. to 1000 ml

E. Preparation of media:
1. Peptone - 6 g
2. Casein hydrolysate of soyabean - 4 g
3. Yeast extract - 3 g
4. Beef extract - 1.5 g
5. Dextrose (dehydrated) - 1 g
6. Agar - 15 g
7. Distilled water sufficient to make 1000ml.

F. Preparation of inoculum:
The peptone water medium was sterilized by autoclaving at 15 Lbs/Sq/inch for 15 minutes. Loop full organisms were transferred from a laboratory maintained culture in to a conical flask (250 ml) containing sterilized peptone water medium. The flask was incubated for 24 hours at 37°C.
G. Sterilization of apparatus required:

Petri dishes, cork borer (8mm), glass syringes and test tubes were sterilized by autoclaving at 15 lbf/in² for 15 minutes.

H. Procedure for microbial assay:

Each conical flask with the medium was cooled to 46⁰C and inoculated with test organism (20 ml of subculture medium per 100ml of the assay medium). To 20 ml each of inoculated media was distributed into petri plates and maintained at room temperature (each reading was taken in triplicate). When media was solidified, four cups (8 mm diameter) were made using sterile cork borer. Two drops of each of the test solutions as well as standard solutions and blank (DMSO) were placed in each cups separately under aseptic condition, the petri plates were kept in the refrigerator for 2 hrs to allow the uniform diffusion of drug into the agar medium. All the petri plates were then incubated at 37⁰C for 24 hours and zones of inhibition (in mm) were measured [15, 16]. The values of zone of inhibition were given in Table 3.

**Anti-fungal activity:**

Method: Cup plate technique was employed in studying the anti-fungal activity.

A. Preparation of standard solutions:

Miconazole nitrate was the reference standard drug prepared in DMSO to get 50μg/ml.

B. Test organisms used were:

1) *Pencillium notatum*
2) *Asperagillus niger* MTCC 277.

C. Preparation of sub culturing media:

1) Peptone – 5.0 g
2) Beef extract – 3.0 g
3) Sodium chloride – 5.0 g
4) Distilled water – q.s. 1000.0ml

D. Preparation of culture medium:

1) Peeled potato – 200-300 g
2) Dextrose – 5 g
3) Agar – 20 g
4) Distilled water – q.s.1000.0ml

E. Preparation of inoculum:

The subculture media was sterilized by autoclaving at 15lbs/sq. inch for 15 minutes. A loop full of organisms was transferred from a laboratory maintained mother culture in to a 25ml conical flask containing sterilized subculture medium. The flask was incubated for 48 hrs at 37⁰C.

F. Sterilisation of apparatus:

Petridishes, glass syringe, cork borer (8mm), conical flask and test tubes were sterilized by autoclaving at 15 lbf/in² for 15 minutes.

G. Procedure for microbial assay:

Each conical flask with the medium was cooled to 46⁰C and inoculated with test organism (20 ml of subculture medium /100 ml of the assay medium). Then 20 ml of inoculated media was distributed into petri dishes. After solidification of the media, four bores are made at equal distance by using a sterile cork borer (8 mm diameter). Into these cups different concentration of standard drug (25 and 50 μg/ml) and different concentrations of quinolinyl pyrazolines derivative (50 and 100 mg/ml) are introduced. Dimethyl sulfoxide was used as a control. After introduction of standard drug and extracts, the plates were placed in a refrigerator at 8-10 C. After two hrs of cold incubation, the petriplates are transferred to incubator and maintained at 37⁰C ± 2⁰C for 48 hrs. After the incubation period, the petriplates were observed for zone of inhibition by using vernier scale. The results evaluated by comparing the zone of inhibition shown by the derivatives with standard drug [17, 18]. The results are the mean value of zone of inhibition measured in millimeters of three sets. The values of zone of inhibition were given in Table 4.

**RESULTS AND DISCUSSION**

Eight novel tetrazole compounds were synthesized by two schemes. The synthesized compounds were characterized by both physical and spectral techniques. The expected functional groups of the compounds were confirmed with their respective peaks in the IR spectra. The nature and number of hydrogens and carbons were confirmed using ¹H and ¹³C NMR spectra respectively. Molecular weight of the compounds was determined by the respective molecular ion peaks from mass spectra. These values were mentioned above under the experimental work.
All the synthesized compounds were screened for antibacterial and antifungal activities. The zone of inhibition results indicate that TZ2, TZ7 and TZ8 have shown significant antibacterial activity whereas; compounds TZ1 and TZ8 have shown significant antifungal activity. Compounds TZ2 and TZ8 were found to exhibit highest activity against *S.aureus* and *B.pimilis*. The compounds TZ2 and TZ7 showed maximum activity against *E.coli*. The compounds TZ1 and TZ7 exhibited most potent activity against *A. niger* and *P. notatum* respectively.

### Table 3: Antibacterial Activity of the Synthesized Compounds.

<table>
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<th>Compound Code</th>
<th>Zone of Inhibition in mm</th>
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<td></td>
<td>50 µg/ml</td>
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<td>TZ-1</td>
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<td>TZ-3</td>
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</tr>
<tr>
<td>(Ethanol)</td>
<td></td>
</tr>
<tr>
<td>MICONAZOLE NITRATE(50µg/ml)</td>
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### Table 4: Antifungal Activity of the Synthesized Compounds.

<table>
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<td>MICONAZOLE NITRATE(50µg/ml)</td>
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**CONCLUSION**

Compounds TZ-4 and TZ-5 were found have moderate activity against Gram +ve and Gram –ve and other compounds are having insignificant activity when compared to standard Streptomycin. And few compounds are screened for anti-fungal activity at concentrations of 50 and 100µg/ml. TZ-1 shows moderate activity on both *Pencillium notatum* and *Aspergillus niger* compared with standard miconazole nitrate. However none of the compounds had shown greater antibacterial and antifungal activities when compared to standard reference streptomycin and miconazole nitrate respectively. Further research is required to develop compounds with better antimicrobial activity.

**ACKNOWLEDGEMENTS**

The authors are grateful to Department of Pharmaceutical Chemistry, Hindu College of Pharmacy, Guntur for providing facilities to perform the research work.

**Authors’ Statements**

**Competing Interests**

The authors declare no conflict of interest.
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