Original Research Article

Synthesis, characterization and antimicrobial activity of novel tetrazoles clubbed with pyrimidine

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ABSTRACT

An attempt was made to synthesize pyrimidine tetrazole derivatives of pharmaceutical interest by oxidative cyclization of chalcones with adequate yield and purity, prompted by the diversity of their wider usage and the fact that they are an integral part of genetic content. The present work involves the reaction of 5-(2,6-dimethylphenyl)-1H-tetrazole with acetic anhydride to yield 1-[5-(2,6-dimethylphenyl)-1H-tetrazol-1-yl] ethanone (1) and which then treated with different aromatic aldehydes in presence of alkaline medium to chalcones (2a-f). Reaction of chalcones (2a-f) with urea and thiourea to produce 5-[5-(2,6-dimethylphenyl)-1H-tetrazol-1-yl]-4-(substituted aryl ) pyrimidin-2-ol (3a-f) and 5-[5-(2,6-dimethylphenyl)-1H-tetrazol-1-yl]-4-(substituted aryl) pyrimidin-2-thiol (4a-f) respectively. All compounds were characterized by infrared spectroscopy (IR), ¹H nuclear magnetic resonance (NMR), and mass spectrometry (MS) to prove the structure and assessed in vitro for their efficacy as antibacterial and antifungal activity against four bacteria. The compounds 3c, 3d and 3f and compounds 4c, 4d and 4f possess very good activity against S. aureus and E. coli and the compounds 3e, 3c and 3a and compounds 4e,4b and 4c possess very good activity against fungi Candida albicans and Aspergillus niger.

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1. Introduction

Since they are a diverse group of natural and synthetic products, many of which have biological applications, nitrogen-containing heterocycles are extremely important. Pyrimidine, which is present in DNA and RNA, has a variety of pharmacological properties, including bactericide, fungicide, vermicide, insecticide, anticancer, and antiviral.¹ Some pyrimidine derivatives reported as Anti-HIV-1 agents,² antileishmanial,³ Anti-inflammatory,⁴ Anticancer,⁵ antimicrobial Antimalarial.⁶ To date, a wide range of pyrimidine and pyrimidine-fused heterocyclic compounds have been documented to have anticancer activity through a variety of mechanisms and targets.⁷–⁹ Pyrimidine derivatives¹⁰¹¹ have played a significant role in the evolution of heterocyclic chemistry and have been widely used as pharmacophores and synths in organic chemistry. A considerable amount of research effort has been centered on these nuclei due to their flexible chemotherapeutic significance. There has been significant progress in this area since the discovery of many synthetic and semi-synthetic antibacterial sulfa products, nitrofuranes, penicillins, cephalosporins, tetracyclines, macrolides, oxazolidinones, and antifungal agents such as fluconazole, ketoconazole, and miconazole, as well as amphotericin B. Despite advancements in antibacterial and antifungal therapies, most antimicrobial drugs still have a long way to go. Antibiotic overuse has
resulted in the emergence of multidrug-resistant microbial pathogens. Pyrimidine based heterocycles are potential bioactive molecules and exhibit antimicrobial, anti-inflammatory, antioxidant, anticancer, antihypertensive and anti-inflammatory. Tetrazole has a great importance as it is bioactive molecules and exhibit antimicrobial, anti-bacterial, anti-inflammatory, antioxidant, anticancer, antihypertensive, anticonvulsant and also act as enzyme inhibitors. Inspired from these facts, in present work an attempt is being made to synthesize pyrimidine’s containing tetrazole and evaluate for antimicrobial activity which has not been reported yet. Hence the present work deals with the reaction of 1-[5-(2,6-dimethylphenyl)-1H-tetrazol-1-yl] ethanone (1) with different aromatic aldehydes in presence of alkaline medium to form (2E)-1-[5-(2,6-dimethylphenyl)-1H-tetrazol-1-yl]-3-(substituted aryl)prop-2-en-1-one derivatives (2a-f).

2. Materials and Methods

Melting points were determined with open capillary. FT-IR spectra were recorded on a Jasco model 4010 spectrophotometer, 1H NMR spectra were recorded in DMSO on a Varian mercury FT-NMR model YH-300 instrument using TMS as internal standard. Mass spectra were recorded on GC-MS auto tune EI instrument.

2.1. Synthetic procedure

2.1.1. General procedure for the preparation

(2E)-1-[5-(2,6-dimethylphenyl)-1H-tetrazol-1-yl]-3-(substituted aryl) prop-2-en-1-one derivatives [2a-f]

A solution of 5-(2,6-dimethylphenyl)-1H-tetrazole (8.5g, 0.05 moles) and heterocyclic aldehydes (0.05 mole) in ethanol (12 ml) was cooled to 5 to 10°C in an ice bath. The cooled solution was treated with drop wise addition of aqueous sodium hydroxide (5 ml, 50%). The reaction mixture was magnetically stirred for 30 min and then left over night. The resulting dark solution was diluted with ice water and carefully acidified using diluted hydrochloric acid. The tetrazole analogues of chalcone which crystallized were collected by filtration after washing with sodium bicarbonate and water. It was further purified by crystallization from ethanol.

2.1.2. Synthesis of 5-[5-(2,6-dimethylphenyl)-1H-tetrazol-1-yl]-4-(substituted aryl) pyrimidin-2-ol derivatives [3a-f]

To a solution of 2E)-1-[5-(2,6-dimethylphenyl)-1H-tetrazol-1-yl]-3-(substituted aryl)prop-2-en-1-one derivatives (2a-f), (0.01mole) in anhydrous ethanol (50 mL), urea (0.01 mole) and aqueous sodium hydroxide (0.01 mole). The reaction mixture was refluxed for 5 hrs and poured into ice cold water the product obtained was filtered, washed with water and crystallized from aqueous ethanol. The purity of the compound was established by TLC using a mixture of hexane and ethyl acetate (7:3) as a mobile phase.

2.1.3. Synthesis of 5-[5-(2,6-dimethylphenyl)-1H-tetrazol-1-yl]-4-(substituted aryl) pyrimidin-2-thiol derivatives [4a-f]

To a solution of 2E)-1-[5-(2,6-dimethylphenyl)-1H-tetrazol-1-yl]-3-(substituted aryl) prop-2-en-1-one derivatives (2a-f), (0.01mole) in anhydrous ethanol (50 mL), thiourea (0.01 mole) and aqueous sodium hydroxide (0.01 mole). The reaction mixture was refluxed for 5 hrs and poured into ice cold water the product obtained was filtered, washed with water and crystallized from aqueous ethanol. The purity of the compound was established by TLC using a mixture of hexane and ethyl acetate (1:1) as a mobile phase.

2.2. Antibacterial and antifungal activity

All the newly synthesized compounds were screened for antimicrobial activity against both gram positive S. aureus and gram negative E. coli bacteria and antifungal activity against C. albicans and A. niger according to cup plate method at a concentration 100ug/0.1ml respectively. Streptomycin and clotrimazole were used as standard for comparison of antibacterial and antifungal activity Indian Pharmacopoeia. Solvent dimethyl sulphoxide (DMSO) was used as control. The results of screening are given in Table 2.
Table 1: agents and conditions: i) Ar-CHO/ EtOH, ii) Urea/Aq. NaOH, iii) Thiourea/Aq. NaOH

| Comp no | R   | Mole. Formula | MW  | % Yield | M.P. °C | Rf  | Found (Calcd) % |
|---------|-----|---------------|------|---------|---------|-----|-----------------|
| 3a      | Fig1| C₁₇H₁₅N₇O₂   | 333  | 72      | 164     | 0.64| (61.20) 4.50 29.39 |
| 3b      | Fig2| C₁₇H₁₄N₆O₂   | 334  | 62      | 172     | 0.65| (61.04) 4.20 25.12 |
| 3c      | Fig3| C₁₈H₁₆N₆O₂   | 348  | 65      | 174     | 0.73| (62.01) 4.60 24.08 |
| 3d      | Fig4| C₁₈H₁₆N₆O₂S  | 364  | 60      | 166     | 0.68| (59.29) 4.38 23.04 |
| 3e      | Fig5| C₁₈H₁₆N₆O₂S  | 364  | 64      | 164     | 0.65| (59.29) 4.38 23.04 |
| 3f      | Fig6| C₁₈H₁₈N₇O₇   | 345  | 74      | 150     | 0.75| (62.57) 4.33 28.36 |
| 4a      | Fig7| C₁₇H₁₄N₇S   | 348  | 72      | 166     | 0.62| (58.41) 4.29 28.03 |
| 4b      | Fig8| C₁₇H₁₆N₆O₂S  | 350  | 66      | 180     | 0.66| (58.22) 4.00 23.95 |
| 4c      | Fig9| C₁₈H₁₆N₆O₂S  | 364  | 68      | 178     | 0.72| (59.26) 4.41 23.05 |
| 4d      | Fig10| C₁₈H₁₆N₆S₂ | 380  | 65      | 166     | 0.68| (56.80) 4.20 22.06 |
| 4e      | Fig11| C₁₈H₁₆N₆S₂  | 380  | 65      | 168     | 0.58| (56.76) 4.22 25.04 |
| 4f      | Fig12| C₁₈H₁₈N₇S   | 361  | 72      | 185     | 0.75| (59.77) 4.14 27.10 |

Table 2: Antibacterial and antifungal data of pyrimidine

| Comp. | Zone of inhibition in mm at 100 μg/0.1ml |
|-------|------------------------------------------|
|       | S. aureus | E. coli | C. albicans | A. niger |
| 3a    | 14      | 12      | 20          | 18       |
| 3b    | 15      | 13      | 20          | 16       |
| 3c    | 18      | 16      | 21          | 15       |
| 3d    | 16      | 14      | 18          | 13       |
| 3e    | 18      | 15      | 21          | 22       |
| 3f    | 20      | 18      | 16          | 12       |
| 4a    | 16      | 13      | 15          | 13       |
| 4b    | 16      | 14      | 20          | 16       |
| 4c    | 18      | 17      | 20          | 14       |
| 4d    | 16      | 15      | 18          | 12       |

Fig. 1: Table1+R+Show 3a

Fig. 2: Table1+R+Show 3b
Fig. 3: Table 1 + R + Show 3c

Fig. 4: Table 1 + R + Show 3d

Fig. 5: Table 1 + R + Show 3e

Fig. 6: Table 1 + R + Show 3f

Fig. 7: Table 1 + R + Show 4a

Fig. 8: Table 1 + R + Show 4b

Fig. 9: Table 1 + R + Show 4c

Fig. 10: Table 1 + R + Show 4d

Fig. 11: Table 1 + R + Show 4e
pyrimidin-2-thiol (4a-f) were synthesized from chalcones of 5-(2,6-dimethylphenyl)-1H-tetrazole. All synthesis steps are presented in scheme 1. The IR spectra shows 1542 (C=N), 1445 (C=C) providing the strong evidence for pyrimidine ring.

1H NMR spectrum shows 7.10-7.58 ppm for aromatic protons and 9.7 ppm for OH protons and 13.4 for SH protons were observed at expected signals.

2.3.1. 3a:5-[5-(2,6-dimethylphenyl)-1H-tetrazol-1-yl]-4-(1H-pyrrol-2-yl)pyrimidin-2-ol
IR: 3745 (OH), 3050 (Ar-CH), 1538 (C=N), 1436 (C=C), 1286 (N=N-N), 1120 and 1145 (Tetrazole ring). 1H NMR: 2.35 (d, 6H, CH3), 6.5-6.8 (d, 1H, CH=CH) 7.30 -8.40 (m, 8H, Ar-H), 9.5 (1H, Ar- OH), Mass spectrum (m/z) molecular ion peak at 332.

2.3.2. 3b: 5-[5-(2,6-dimethylphenyl)-1H-tetrazol-1-yl]-4-(furan-2-yl)pyrimidin-2-ol
IR: 3744 (OH), 3054 (Ar-CH), 1536 (C=N), 1432 (C=C), 1280 (N=N-N), 1120 and 1145 (Tetrazole ring). 1H NMR: 2.35 (d, 6H, CH3), 6.5-6.8 (d, 1H, CH=CH) 7.30 -8.40 (m, 7H, Ar-H), 9.5 (1H, Ar- OH), Mass spectrum (m/z) molecular ion peak at 334.

2.3.3. 3c: 5-[5-(2,6-dimethylphenyl)-1H-tetrazol-1-yl]-4-(5-methylfuran-2-yl)pyrimidin-2-ol
IR: 3742 (OH), 3058 (Ar-CH), 1538 (C=N), 1432 (C=C), 1282 (N=N-N), 1245 (-OCH3), 1120 and 1145 (Tetrazole ring). 1H NMR: 2.35 (d, 9H, CH3), 6.5-6.8 (d, 1H, CH=CH) 7.30 -8.40 (m, 7H, Ar-H), 9.5 (1H, Ar- OH), Mass spectrum (m/z) molecular ion peak at 334.

2.3.4. 3d: 5-[5-(2,6-dimethylphenyl)-1H-tetrazol-1-yl]-4-(5-methylthiophen-2-yl)pyrimidin-2-ol
IR: 3740 (OH), 3058 (Ar-CH), 1546 (-NO2), 1530 (C=N), 1441 (C=C), 1278 (N=N-N), 1120 and 1145 (Tetrazole ring). 1H NMR: 2.35 (d, 9H, CH3), 6.5-6.8 (d, 1H, CH=CH) 7.30 -8.40 (m, 7H, Ar-H), 9.5 (1H, Ar- OH), Mass spectrum (m/z) molecular ion peak at 364.
The antibacterial activity was evaluated by zone of inhibition method at 100 μg/0.1ml concentration. The results of antibacterial activity were compared with standard drug ciprofloxacin. Most of the synthesized compounds showed antibacterial activity against the tested Bacteria. It is evident that most of the compounds are very weakly active and few are moderately active against *S. aureus* and *E. coli* but compounds 3c, 3d and 3f and compounds 4c, 4d and 4f possess very good activity against *S. aureus* and *E. coli* at concentration of 100μg/0.1ml.

Similarly, the results of preliminary antifungal bioassays were compared with standard drug clotrimazole. Most of the synthesized compounds showed antifungal activity against the tested fungi. It is evident that the compounds 3e, 3c and 3a and compounds 4e, 4b and 4c possess very good activity against fungi *Candida albicans* and *Aspergillus niger* at concentration of 100μg/0.1mL. Compound 3d and 4f showed moderate activity all bacteria and fungi tested.

### 4. Conclusion

Tetrazole and Pyrimidine an important group of heterocyclic compounds reported to have different biological activities and hence the present studies were undertaken in order to synthesize Tetrazole clubbed with pyrimidines in order to potentiate the combined therapeutic effect of both heterocyclic compounds. So all the synthesized compounds were investigated them for their antibacterial and antifungal activity. Compounds with thiopene, furan and pyrrole substituents on pyrimidine clubbed with Tetrazole exhibited significant antibacterial and antifungal activity when compared with control. The compounds with pyrimidines substituents groups showed significant activity when compared to standard drug ciprofloxacin and clotrimazole respectively.

### 5. Source of Funding

None.

### 6. Conflict of Interest

None.

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