Synthesis of New 1,2,4-Triazole[3,4-b][1,3,4]thiadiazoles Bearing Pyrazole as Potent Antimicrobial Agents

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A new series of 6-(aryl/hetaryl)-3-(5-methyl-1-phenyl-1H-4-pyrazolyl)[1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles (7a—j) has been synthesized by the reaction of 4-amino-5-(5-methyl-1-phenyl-1H-4-pyrazolyl)-4H-1,2,4-triazol-3-yl-hydrosulfide (6) with POCl3 and the corresponding aryl/hetaryl carboxylic acid, in ethanol at reflux temperature for 12 h. All the synthesized compounds were tested for in vitro activities against certain strains of bacteria such as Staphylococcus aureus, Bacillus subtilis, Escherichia coli and fungi such as Aspergillus niger, Aspergillus nodulans, Alternaria alternate. Compounds having 4-chlorophenyl (7d), 4-aminophenyl (7f), 4-nitrophenyl (7b) and 3-pyridyl (7i) substituents at 6-position of thiadiazole ring, showed marked inhibition of bacterial and fungal growth nearly equal to the standards. The other new compounds also showed appreciable activity against the test bacteria and fungi.

Key words fused-triazole; antibacterial agent; antifungal agent; antimicrobial agent

Pyrazole and its derivatives represent one of the most active classes of compounds possessing a wide spectrum of biological activities, including antibacterial,1 anti-fungal,2 her-bicidal,3 insecticidal4 and other biological activities.5—7 Similarly, the biological activities of various 1,2,4-triazole derivatives and their N-bridged heterocyclic analogs have been widely investigated as antitumor,8,9 antiviral,10 anti-inflammatory,11 analgesic12 and antidepressant.13 1,2,4-Triazole system is also an important starting material in the synthesis of biologically active heterocycles, which constitute an important class of organic compounds with diverse biological activities, including antiparasitic, analgesic, antibacterial and anti-inflammatory activities.13—18 Further, triazole fused with other heterocyclic rings is also found to possess diverse applications in the field of medicine.19—22 The commonly known systems are triazole-pyridines,18,20 triazole-pyridazines,20 triazole-pyrimidines,21 triazole-pyrazines,22 triazole-triazines21 and triazole-thiadiazines.23 In addition, it has been reported that thiadiazoles exhibit a broad spectrum of biological effectiveness such as anti-parkinsonism,24 hypogly-caemic,25 anticancer,26 anti-inflammatory,27 anti-asthmatic28 and anti-hypertensive29 activities.

Inspired by the biological profile of pyrazole, triazole, thiadiazole, and in continuation of our research on biologically active heterocycles,30—35 it was thought worthwhile to synthesize some new heterocyclic compounds containing pyrazole, triazole and thiadiazol rings in one molecular framework. We report herein the synthesis of a new series of 1,2,4-triazole[3,4-b][1,3,4]thiadiazoles bearing pyrazole and their antimicrobial activity.

Results and Discussion

Synthesis The starting material, (E)-2-acetyl-3-(dimethylamino)-2-propenoate (2), was obtained via condensation of ethyl acetoacetate (1) with N,N-dimethyl-dimethoxymethanamine,36 which on cyclo-condensation with phenyl hydrazine resulted 5-methyl-1-phenyl-1H-4-pyrazolecarboxylate (3).36 The 5-methyl-1-phenyl-1H-4-pyrazolecarboxyhydrazide (4) was obtained in 72% yield via hydrazinolysis of compound 3 with hydrazine hydrate. The hydrazide 4 on reaction with carbon disulfide and potassium hydroxide, in ethanol, followed by acidification gave 5-(5-methyl-1-phenyl-1H-4-pyrazolyl)-1,3,4-oxadiazol-2-yl-hydrosulfide (5) in 70% yield. Reaction of 5 with hydrazine hydrate under reflux for 6 h resulted 4-amino-5-(5-methyl-1-phenyl-1H-4-pyrazolyl)-4H-1,2,4-triazol-3-yl-hydrosulfide (6) in 69% yield. The final compounds, 6-(aryl/hetaryl)-3-(5-methyl-1-phenyl-1H-4-pyrazolyl)[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (7a—j), were synthesized in 66—78% via the reaction of 6 with POCl3 and the corresponding aryl/hetaryl carboxylic acids, in ethanol at reflux temperature for 12 h (Chart 1). The structures of all the newly synthesized compounds were confirmed by their elemental analyses, electron ionization (EI) mass, IR, 1H- and 13C-NMR spectral data.

In the IR spectra of 7a—j, appearance of bands at 1610 (C=O), 1520 (C=N) cm⁻¹ and the absence of NH2 and SH stretching vibrations provided the evidence of biological effectiveness as anti-parkinsonism,29 hypoglycaemic,25 anticancer,26 anti-inflammatory,27 anti-asthmatic28 and anti-hypertensive29 activities.

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followed by the presence of aromatic protons in the region of δ 7.60 and 7.92 ppm well support the structures. In the 1H-NMR spectra, the prominent signals corresponding to the carbons of [1,2,4]triazolo[3,4-b][1,3,4]thiadiazole ring for all the compounds, observed nearly at δ 152.1, 144.3 and 134.9 ppm are proof of further evidence of their structures. In summary all the newly synthesized compounds exhibited satisfactory spectral data consistent with their molecular structures.

Biological Properties. Antibacterial Activity The newly synthesized compounds 7a—j were tested for their in vitro antibacterial activity against Staphylococcus aureus, Bacillus subtilis and Escherichia coli by using the agar disc-diffusion method. The results of the preliminary antimicrobial testing of the prepared compounds, the typical broad spectrum of antibacterial drug ciprofloxacin are shown in Table 1.

Among the tested compounds, four compounds showed considerable activity almost equal to the activity of ciprofloxacin. The other compounds were found to be moderate or least effective. In order to get some meaningful results, the structure–activity relationship was carried out. From the bacterial screening results it has been observed that the compounds having 4-chlorophenyl (7d), 4-aminophenyl (7f), 4-nitrophenyl (7h) and 3-pyridyl (7i) substituents at 6-position of thiadiazole ring, showed marked inhibition of bacterial growth. Compounds having phenyl (7a), 4-methylphenyl (7b) and 4-methoxyphenyl (7c) substituents at 6-position of thiadiazole ring showed least activity; whereas the compounds having 2-chlorophenyl (7e), 4-hydroxyphenyl (7g) and 4-pyridyl (7j) substituents at 6-position of thiadiazole ring showed moderate effect on the growth of bacteria. The comparison of growth inhibition zone diameter (mm) for the selected compound 7 and standard drug against different bacteria is presented in Fig. 1.

Antifungal Activity All the newly synthesized compounds 7a—j were also screened for their antifungal activity against Aspergillus niger, Aspergillus nodulans and Alternaria alternate by food poison technique. The results of the preliminary antifungal testing of the prepared compounds, the typical broad spectrum of the potent antifungal drug amphotericin B are shown in Table 1. The antifungal activity data reveal that compounds containing 4-chlorophenyl (7d), 4-aminophenyl (7f) and 3-pyridyl (7i) substituents at 6-position of thiadiazole ring, are showing excellent activity against the test fungi and nearly equal to the standard amphotericin B.

Conclusion
In conclusion, a new series of 1,2,4-triazolo[3,4-b][1,3,4]-thiadiazoles bearing pyrazoles 7a—j has been synthesized and evaluated for their antimicrobial activity. Most of the new compounds showed appreciable antimicrobial activity. Among them, the compounds having 4-chlorophenyl (7d), 4-aminophenyl (7f), 4-nitrophenyl (7h) substituents at 6-position of thiadiazole ring showed marked inhibition of bacterial and fungal growth nearly equal to the standards.

Experimental
General All the reagents used were of commercial grade. Reactions were monitored by thin-layer chromatography (TLC) on pre-coated silica gel F254 plates obtained from Merck and the compounds visualized either by exposure to UV light or dipping in 1% aqueous potassium permanganate solution. Melting points were determined through a Fisher–Johns apparatus and are uncorrected. IR spectra were recorded using KBr disk on a Perkin–Elmer FTIR spectrometer. The 1H- and 13C-NMR spectra were recorded on a Varian Gemini spectrometer (300 MHz for 1H and 75 MHz for 13C-NMR). Chemical shifts are reported in δ ppm units with respect to tetramethyl silane (TMS) as internal standard and coupling constants (J) are reported in Hz units. Mass spectra were recorded on a VG micro mass 7070H spectrometer. Elemental analyses (C, H, N), determined by means of a Perkin–Elmer 240 CHN elemental analyzer, were within ±0.4% of theory.

Typical Procedure 5-Methyl-1-phenyl-1H-4-pyrazolecarboxyhydrazide (4) A mixture of compound 3 (5 mmol) and hydrazine hydrate (5 mmol) in ethanol (15 ml) was refluxed for 4 h, cooled to room temperature and filtered. The crude product was recrystallized from ethanol to give new intermediate 4 as white crystal. Yield 72%, mp 162—164 °C. IR (KBr) cm⁻¹: 3269, 3062, 2933, 1660, 1510. 1H-NMR (CDCl₃) δ: 2.61 (s, 3H, CH₃), 5.49 (s, 2H, NH), 7.15—7.25 (m, 5H, Ar-H), 8.26 (s, 1H, Ar-H), 8.96 (s, 1H, NH). 13C-NMR (CDCl₃) δ: 258 (M⁺). Anal. Calcd for C₁₁H₁₀N₄O: C, 66.70; H, 5.59; N, 26.16. Found: C, 66.90; H, 5.59; N, 26.05.

Table 1. Antimicrobial Activity of Compounds (7a—j)

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<th>Compound</th>
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<tr>
<td>Ciprofloxacin</td>
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<tr>
<td>Amphotericin B</td>
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The compounds 7a—j and the standards used were of 100 μg/8 mm discs.

Fig. 1. Comparison of Antibacterial Activity (Growth Inhibition Zone Diameter, mm) of Selected Compounds with Ciprofloxacin (CF)
4-(Methyl-1-phenyl-1H-4-pyrazolyl)-6-(4-pyridyl)[1,2,4]triazolo[3,4-b][1,3]thiadiazole (7a—j) was obtained by reacting compound 6 (5 mmol) in POCI₃ (10 ml), a solution of aryl/heterocyclic acid (5 mmol) in ethanol (15 ml) was added and the reaction mixture was heated under reflux for 12 h under anhydrous conditions. The solvent was distilled in vacuo, the residual mass was poured over crushed ice and the excess POCI₃ was neutralized with 10% sodium bicarbonate solution. The solid thus separated was filtered, washed with 10% sodium bicarbonate solution and finally with water, and dried and recrystallized from ethanol to give pure compounds.

3-(5-Methyl-1-phenyl-1H-4-pyrazolyl)-6-(4-pyrrolidinyl)-1-[2,4,5,6-tetrahydro-1H-pyridin-4-yl]pyrazolo[1,2,4]triazol-3-yl-hydrosulfide (6)

This was obtained by reacting compound 6 (5 mmol) with 4-nitrobenzoic acid (0.66 g) as described in the typical procedure. Yield 68%, mp 142—144°C. IR (KBr) cm⁻¹: 3300, 2928, 1612, 1514, 1350. 1H-NMR (CDCl₃) δ: 1.30 (3H, CH₃), 2.49 (3H, CH₃), 7.15—7.25 (5H, 5H, Ar-H), 8.05 (s, 1H, Ar-H). 13C-NMR (CDCl₃) δ: 14.7, 119.8, 125.1, 127.9, 130.9, 132.4, 137.6, 138.6, 142.0, 146.9. MS m/z: 273 (M⁺). Anal. Calc. for C₂₀H₁₆N₆S: C, 64.50; H, 4.33; N, 22.56. Found: C, 64.42; H, 4.30; N, 22.52.

Typical Procedure (Aryl/Heterocyclic-3-(5-methyl-1-phenyl-1H-4-pyrazolyl)-6-(4-pyrrolidinyl)-1-[2,4,5,6-tetrahydro-1H-pyridin-4-yl]pyrazolo[1,2,4]triazol-3-yl-hydrosulfide (6)

This was obtained by reacting compound 6 (5 mmol) in POCI₃ (10 ml), a solution of aryl/heterocyclic carboxylic acid (5 mmol) in ethanol (15 ml) was added and the reaction mixture was heated under reflux for 12 h under anhydrous conditions. The solvent was distilled in vacuo, the residual mass was poured over crushed ice and the excess POCI₃ was neutralized with 10% sodium bicarbonate solution. The solid thus separated was filtered, washed with 10% sodium bicarbonate solution and finally with water, and dried and recrystallized from ethanol to give pure compounds.

References and Notes