Spiro-heterocyclics: A convenient synthesis and antimicrobial activity of 3-(5-substituted phenyl-1,3,4-thiadiazole-2-yl)-5,8-dithiaspiro[3,4]octan-2-ones and 1,4-dithia-6-azaspiro[4,4]-nonan-7-ones

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A new series of novel 3-(5-substituted phenyl-1,3,4-thiadiazole-2-yl)-5,8-dithiaspiro[3,4]octan-2-ones and 1,4-dithia-6-azaspiro[4,4]nonan-7-ones have been synthesized from a common intermediate, in good yields. These compounds have been screened for their antibacterial and antifungal activity against different pathogenic strains of bacteria and fungi. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) have been determined for the test compounds as well as for reference standards. Compounds 4b, 4c, 4d, 5b, 5c, and 5d have shown good antibacterial activity whereas compounds 4b, 4d, 5b, and 5d have displayed better antifungal activity.

Keywords: Substituted phenyl, 1,3,4-thiadiazole, dithiaspiro, azaspiro, antimicrobial antibacterial activity, antimicrobial antifungal activity

Efficient synthesis of drug-like small molecules has been interest for medicinal chemists and chemical biologists because, this provide the important scaffolds in fewer steps and these molecules play very important role in drug discovery processes. Several bacterial infections such as diarrhea, food poisoning, rheumatic salmonellosis, extraintestinal and intestinal wall infections are caused by gram-positive and gram-negative pathogens. The resistance of pathogens bacteria towards available antibiotics is rapidly becoming a major threat to human health worldwide. In addition, fungal infections continue to increase dramatically because of growing number of immunocompromised hosts such as AIDS patients or those undergoing anticancer chemotherapy and transplantation. Resistance to know antibiotics is becoming great concern in scientific community and big challenge to develop new scaffold as biologically active molecules. Therefore, design of new antimicrobial compounds to deal with these problems is of prime interest.

1,3,4-Thiadiazoles are a class of heterocycles which have attracted significant interest in medicinal chemistry and they have a wide range of pharmaceutical and biological activities including antibacterial, antifungal, antitubercular, analgesic and leishmanicidal agents. A large number of spiro heterocyclic compounds were prepared and their applications investigated. β-Lactams (azetidin-2-ones) play a neutral role in medicinal chemistry as a key intermediate for the synthesis of penicillin and its analogues. Also the azolidinone derivatives are used as biological agents such as bactericidal, fungicidal and insecticidal.

In light of above literature and abundance on bio-potentials of spiro heterocycle analogues, we designed two or more different heterocyclic moieties in a single molecule and were confident that these framework would provide the important structural motifs for the discovery of new antimicrobial agents. In continuation of our research on efficient synthesis of biologically active small molecules, we developed spiro-heterocycles containing 1,3,4-thiadiazole with dithiaspiro/azaspiro derivatives and demonstrated their antimicrobial activity. The structures of these compounds was established by the IR, 1H NMR spectral data and elemental analysis (Scheme I).

The required starting material 2-amino-5-substituted aryl-1,3,4-thiadiazoles was prepared by the known method. A mixture of phenyl thiosemicarbazide was stirred in conc. H2SO4 below 15°C to stand for an hour and then poured into cold water. The product which was precipitated on neutralization with ammonia solution was filtered, washed with...
water and recrystallized from ethanol to furnish the corresponding thiadiazole derivatives.

Antibacterial Activity

The synthesized pure compounds were screened for antibacterial and antifungal activities adopting standard protocols\(^3\). The antibacterial activity of prepared final pure compounds 4a-d and 5a-d was performed against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Klebsiella pneumoniae* using ciprofloxacin as positive and DMSO as negative control. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined and the activity was reported in \(\mu g/mL\). The nutrient broth, which contained logarithmic serially two fold diluted amount of test compounds and controls were inoculated with approximately \(5 \times 10^5\) c.f.u./mL of activity dividing bacteria cells. The cultures were incubated for 24 h at 37°C and the growth was monitored visually and spectrophotometrically. The antibacterial results were summarized in Table I that compounds 4b and 4c showed moderate activity against gram-positive bacteria *S.aureus* and *B.subtilis* where as 5b, 5c and 5d were active against gram-negative *K.pneumoniae* ranging from 30 to 50 \(\mu g/mL\) concentration and only one compound 4d was found active against *E.coli* 25 \(\mu g/mL\) concentration. MBC/MIC ratio of all active compounds is ranging from 2.0 to 3.3 suggesting these compounds are bactericidal not bacteriostatic. Antimicrobial agent is considered bacteriostatic when the minimal MBC/MIC ratio is greater than or equal to 8 whereas it is considered bactericidal if MBC/MIC ratio is less than or equal to 4.

Fungicidal Activity

The antifungal activity, of the prepared pure compounds was performed against *Tilletia indica*, *Trichoderma*, *P.cubensis*, *S.fuliginea* and *P.infestans* using Griseofulvin as positive and DMSO as negative control. Minimum inhibitory concentration (MIC) was determined and reported in \(\mu g/mL\). Antifungal activity was carried out through disk diffusion method\(^3\). All fungal cultures were routinely maintained...
on Sabouraud dextrose (SDA) and incubated at 28°C. The antifungal activities are summarized in Table II, only for those compounds which were found active against any of these strains of fungi. It is inferred from Table II that compounds 4b, 4d, 5b and 5d showed antifungal activity against T.indica, Trichoderma P.cubensis, S.fuliginea and P.infestans strain of fungi ranging from 10 to 20 μg/mL in concentration which is comparable to Griseofulvin.

The structure-activity relationship (SAR) of the tested compounds for antimicrobial activity can be summarized as follows:

(i) In the series of compounds 4a-d, the substitution at phenyl ring and presence of β-Lactam moiety. However in the compounds 5a-d, the substitution at phenyl ring and presence of thiazolidinone moiety is an important scaffold for better antibacterial as well as antifungal activity.

(ii) Most of the antibacterial compounds have MBC/MIC ratio below 4, it means they are bactericidal not bacteriostatic, Antifungal activity of these compounds was similar or ever better in some cases as compare to Griseofulvin, a known antifungal agent. The possible mechanism for the antibacterial activity of examined compounds is not known at the moment and investigations are being done to investigate the mechanism of antibacterial action, and to synthesize more effective compounds.

**Experimental Section**

Melting points were recorded in Richerf Thermover instrument and are uncorrected. The IR spectra were recorded on Perkin-Elmer RXI spectrometer in KBr. ¹H and ¹³C NMR were recorded on Bruker-300 and Bruker Avance II 400 spectrometer using tetramethylsilane (TMS) as an internal standard and DMSO-d6/CDCl3 as solvent. The micro analytical data were collected on Elemental Vario EL III elemental analyzer. All chemicals used were purchased from Merck and Fluka chemicals. The homogeneity of compounds was checked by thin layer chromatography (TLC) on glass plates coated with silica gel G254 (Merck, Mumbai, India) using chloroform-methanol (3:1) mixture as mobile phase.

**General procedure for synthesis of 5-substituted phenyl-N-(1,3-dithiolan-2-ylidene)-1,3,4-thiadiazole-2-amime (R=H)**

A mixture of 2-amino-5-phenyl-1,3,4-thiadiazole (0.1 mol) in methanol was stirred for 2 h. The resulting 5-phenyl-2-disodium dithiocarbamate 1,3,4-thiadiazole was treated with excess of 1,2-dichloroethane for 1h. Excess of solvent was removed and resulting mass was poured into water, filtered and washed with water. The product obtained
was purified by recrystallization from ethanol to get crystalline solid products 3a-d.

3a: Yield 64%. m.p. 155°C. IR (KBr): 3060 (C-H aromatic ring stretching), 1590 (C=N stretching), 1460, 1240, 1060 cm⁻¹ (C-S-C stretching); ¹H NMR (DMSO-d₆): δ 4.1 (s, 4H, CH₂), 6.5 (s, 1H, CH), 6.8-7.9 (m, 4H, aromatic ring); ¹³C NMR (DMSO-d₆): δ 35.6, 126.7, 127.7, 129.2, 134.4, 166.5, 184.0.

3b: Yield 69%. m.p. 172°C IR (KBr): 2950 (C-H aromatic ring stretching), 1560 (C-N stretching), 1470 (C=C stretching), 1245, 1050 (C-S-C stretching) and 810 cm⁻¹ (C=S-C stretching), 1245, 1015 (C-S-C stretching), 815 (C-CH₃ stretching), and 810 cm⁻¹ (C=S-C stretching); ¹H NMR (DMSO-d₆): δ 3.9 (d, 4H, CH₂), 6.9-7.3 (m, 5H, aromatic ring); ¹³C NMR (DMSO-d₆): δ 35.6, 127.2, 128.3, 131.4, 132.0, 147.0, 185.1, 182.5.

3c: Yield 68%. m.p. 190°C. IR (KBr): 3160 (C-H aromatic ring stretching), 1560 (C=N stretching), 1245, 1050 (C-S-C stretching) and 810 cm⁻¹ (C-NO₂ stretching); ¹H NMR (DMSO-d₆): δ 3.5 (d, 4H, CH₂), 6.6-7.0 (m, 5H, aromatic ring); ¹³C NMR (DMSO-d₆): δ 36.6, 56.8, 115.7, 116.4, 117.6, 159.9, 163.1, 184.5.

3d: Yield 72%. m.p. 166°C. IR (KBr): 3160 (C-H aromatic ring stretching), 1550 (C=N stretching), 1560 (C=N stretching), 1240, 1020 (C-S-C stretching), and 810 cm⁻¹ (C-CH₃ stretching); ¹H NMR (DMSO-d₆): δ S 3.6 (d, 4H, CH₂), 6.6-7.0 (m, 3H, aromatic ring); ¹³C NMR (DMSO-d₆): δ 22.3, 37.2, 121.7, 127.6, 128.2, 132.4, 165.6, 184.0.

General procedure for synthesis of 3-(5-substituted phenyl)-1,3,4-thiadiazol-2-yl)-5,8-dithiaspiro [3,4] octane-2-one (R=H)

A mixture of 5-phenyl-2-(imino-1,3-dithiolan-2-yl)-1,3,4-thiadiazole (0.01 mol) and mercapto acetic acid (0.1 mol) was refluxed in dioxane for 2 h. The resulting solution was poured into water and neutralized with ammonia solution. The solid compound thus obtained was filtered, dried and recrystallized from ethanol to get crystalline solid products 5a-d.

5a: Yield 66%. m.p. 235°C. IR (KBr): 3010 (C-H aromatic stretching), 1610 (C=N stretching), 1460 (C=C stretching) and 1230, 1020 cm⁻¹ (C-S-C stretching); ¹H NMR (DMSO-d₆): δ 7.4-7.9 (d, 4H, H-aromatic), 5.0 (s, 2H, CH₂ proton), 4.4 (s, 2H, CH₂ proton), 2.3 (s, 3H, CH₃); ¹³C NMR (DMSO-d₆): δ 22.3, 36.7, 58.7, 62.5, 75.2, 120.6, 126.0, 127.7, 163.5, 164.6, 198.0.

5b: Yield 69%. m.p. 220°C. IR (KBr): 3010 (C-H aromatic stretching), 1560 (C=N stretching), 1225, 1015 cm⁻¹ (C-S-C stretching); ¹H NMR (DMSO-d₆): δ 8.2 – 8.3 (d, 4H, H-aromatic),
2.6 – 2.7 (d, 2H, CH₂ proton), 2.5 – 2.6 (d, 2H, pyrrolidin ring proton); ¹³C NMR (DMSO-δ₆): δ 25.4, 29.2, 35.6, 82.2, 128.4, 130.6, 132.0, 147.5, 164.01, 169.5, 174.0.

5c: Yield 64%. m.p. 245°C. IR (KBr): 3015 (C-H stretching), 1590 (C≡N stretching), 1450 (C=C ring proton); ¹³C NMR (DMSO-δ₆): δ 25.4, 28.7, 36.2, 54.9, 82.4, 113.4, 114.6, 117.6, 160.4, 164.0, 169.2, 174.4.

Conclusion

In the present investigation, a series of new spiro heterocycles have been synthesised and screened for their antifungal and antibacterial activity. The activity reveals that the synthesized compounds possess moderate to good activity profile. The insights gained from this study will be useful for development of new anti-infective agents.

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References
