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# Synthesis, Characterization and Antimicrobial Evaluation of Some 5-(Substituted)-2-Amino-Thiadiazoles.

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## ABSTRACT

Novel Thiadiazoles were synthesized from 4-hydroxybenzoic acid and thiosemicarbazide.From these compounds various derivatives of 1,3,4-Thiadiazole derivatives (C1-C5) have been synthesized. The chemical structures of the synthesized compounds were confirmed by means of IR, $^{1}$H NMR, and nitrogen estimation. These compounds were screened for antibacterial (*Staphylococcus aureus* ATCC 9144*, Becillus Cereus* ATCC *11778, Escherichia coli* ATCC 25922*, Pseudomonas aeruginosa* ATCC 2853 and anti-fungal (*Aspergillus niger* ATCC 9029 and *Aspergillus fumigatus* ATCC 46645) by paper disc diffusion technique.

**Keywords:** 1,3,4-Thiadiazole; Synthesis; Antibacterial; Antifungal; NMR.

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## INTRODUCTION

Pathogenic bacteria are one of the major causes of human death and disease. Pathogenic bacteria cause infectious diseases like tetanus, typhoid fever, diphtheria, syphilis, cholera, food borne allergies, leprosy and tuberculosis[1-3]. Therefore nowadays it is necessary to explore new compounds with potential effects against pathogenic bacteria. There are some organic materials especially heterocyclic compounds which can play an important role in regulating bacterial activities. Among the heterocyclic compounds thiazoles and the derivatives of thiazoles are a group of organic species which shows remarkable antimicrobial activities. Thiazoles and pyrazoles have been reported to show pharmacological activities. Some of them are used as medi-cines [4]. According to literature survey, thiazoles were re-ported to possess antimicrobial[5-8], cardiotonic[9], fungicidal[10],sedative[11], anaesthetic[12], bactericidal[13] and antiinflammatory[14]. The synthesis of thiazole derivatives is important for their wide range of pharmaceutical and biological properties. One classical and widely used method is the condensation of $\alpha$-haloketones with thioamides, known as the Hantzsch reaction [15-17]. Another efficient method is the introduction of substitution onto a thiazole core structure through Stille coupling [18]. Above observation prompted us to synthesize the title compounds (C1-C5) with presumption that drug intermediates based on thiosemicarbazide with various amines would produce novel thiadiazole derivatives with potent biological activities. Their chemical structure was confirmed by IR, $^1$HNMR, and nitrogen estimation. These compounds were screened for their antibacterial activity against two gram + ve bacteria (*Staphylococcus aureus* ATCC 9144, and two gram - ve bacteria *Escherichia coli* ATCC 25922*,* and anti-fungal (*Aspergillus niger* ATCC 9029 and *Aspergillus fumigatus* ATCC 46645) activities by paper disc diffusion technique.

## EXPERIMENTAL

All chemicals used in this study were purchase from Aldrich Chemicals and were used without further purification. All melting points were taken in open capillary tube and are uncorrected. The purity of the compounds was checked by TLC on pre-coated $SiO_2$ gel ($HF_{254}$, 200 mesh) aluminum plates (E Merk) using Butanol: Acetic acid: water (4:1:5) visualized in iodine chamber. FTIR spectra were recorded with Perkin Elmer spectrophotometer. The $^1$HNMR spectra were determined with Brucker 400 MHz FTNMR spectrometer.

### Synthesis of – (C): 4-(5-amino-1,3,4-thiadiazol-2-yl)Phenol

A mixture of thiosemicarbazide (9.11 g, 0.1 mol),aryl 4-hydroxy benzoic acid (13.8 g,0.1 mol), and conc. Sulphuric acid (5 ml) in 50 ml of ethanol was refluxed for 1.5 hour and poured onto crushed ice. The solid separated out was filtered, washed with cold water and recrystallized from ethanol to separate the first step product. The reaction scheme is shown in figure-1.

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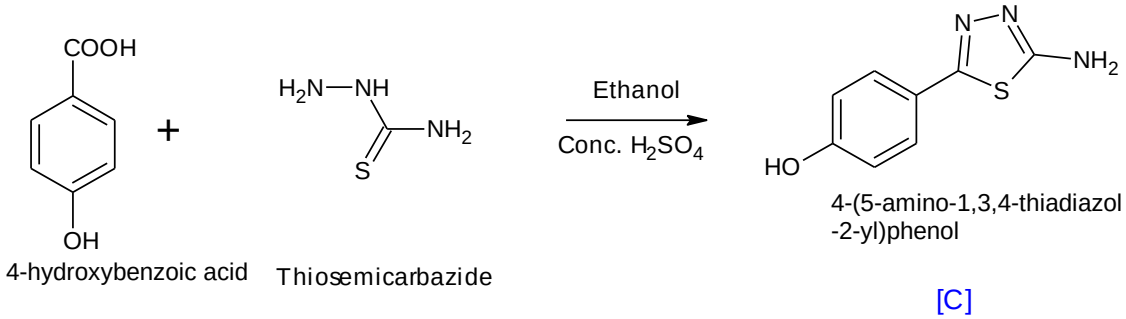
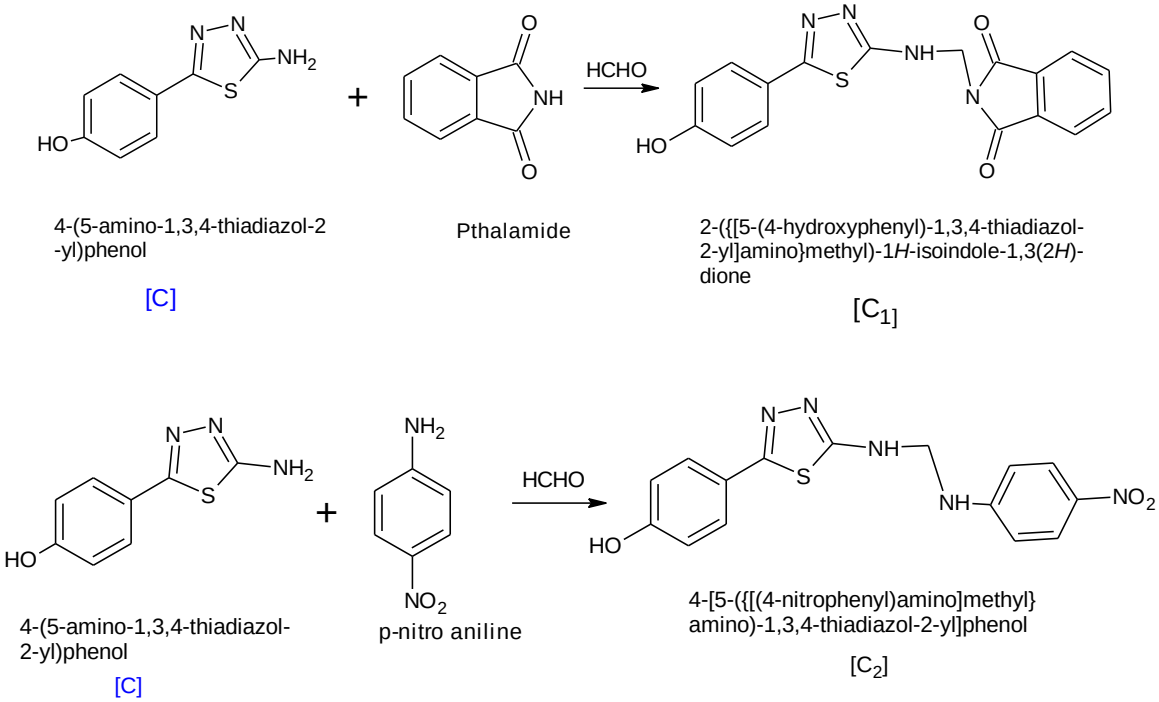



**Figure-1: Synthesis of 4-(5-amino-1,3,4-thiadiazol-2-yl)Phenol.**

**General method for synthesis of $C_1 – C_5$:**

A methanolic solution of first step product (2 gm, 0.001mole) was charged into a three nack flask equipped with a stirred and dropping funnel. The solution was stirred to dissolve it completely. To this methanolic solution, formaldehyde (7 ml, 37%) was added dropwise during 15-20 minutes. The resulting mixture was stirred during half an hour to complete reaction of formaldehyde and to yield methylol derivative. To this reaction mixture, the methanolic solution of amine (1.5 gm,0.001 mol) was added dropwise with stirring in about half an hour at 30 $^{o}$ C temperature and refluxed for two hour at 65-70 $^{o}$C. it was allowed to cool and poured in ice water. The solid obtained was filtered off washed thoroughly with hot water and air dried. The reaction scheme for these compounds are shown in figure-2.



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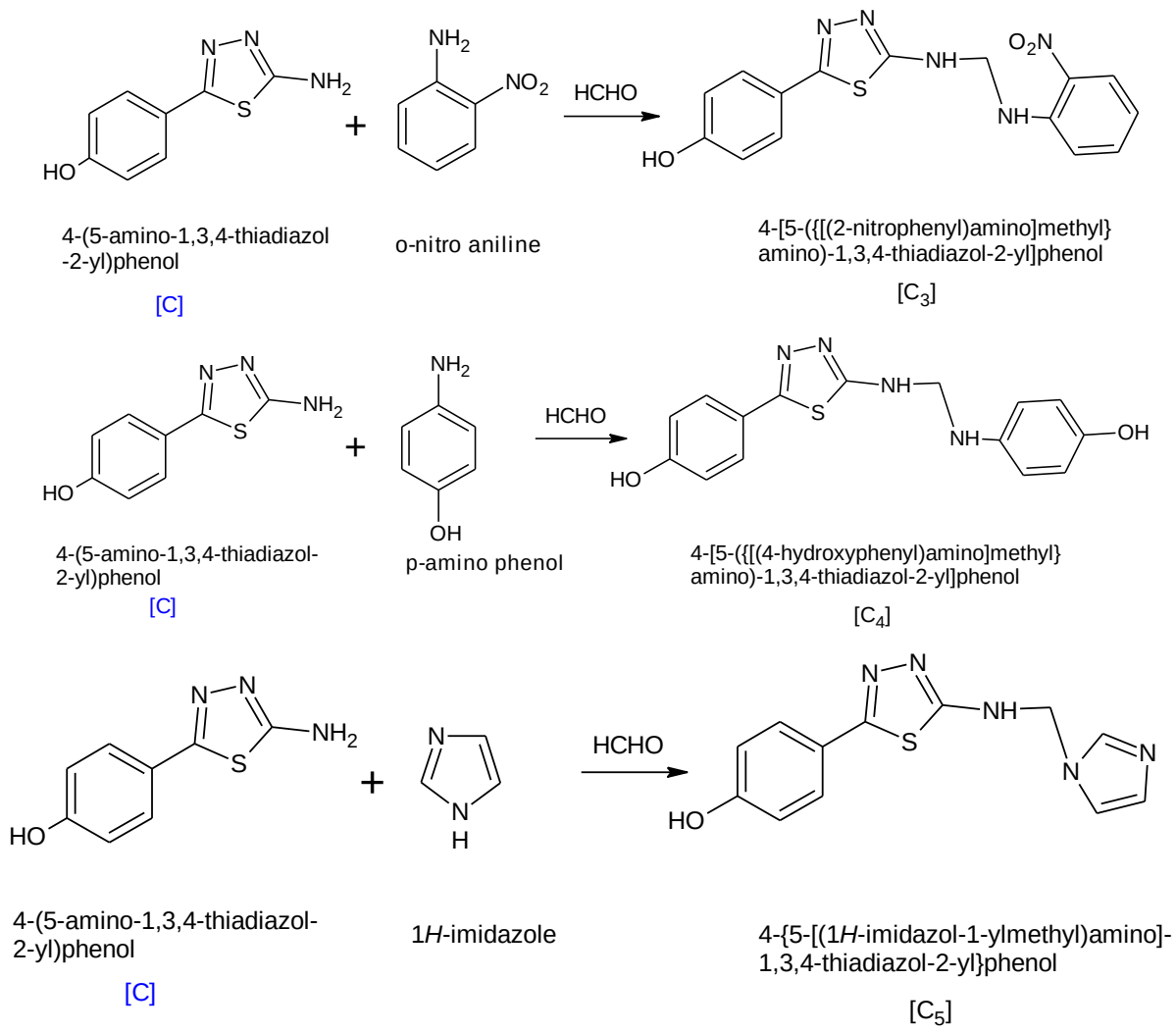


**Figure-2: Synthesis of 5-(substituted)-2-amino-thiadiazoles.**

The data of physical characteristics of synthesized compounds are shown in table-1. Percentage of nitrogen was estimated by Kjeldahl method. All the synthesized compounds were characterized by IR and $^{1}$H NMR. The spectral data of synthesized compounds are shown in table-2.

**Table 1: Characterization Data of Synthesized Compounds.**

| Compd. | Molecular Formula | Melting Point $^{o}$ C | Yield (%) | Mol. Wt gm/mole | Nitrogen (%) | |
|---|---|---|---|---|---|---|
| C | $C_8H_7N_3OS$ | 140 | 68 | 193 | 16.37 | 15.68 |
| C1 | $C_{17}H_{12}N_4O_3S$ | 108 | 65 | 342 | 21.14 | 19.08 |
| C2 | $C_{14}H_{15}N_5O_2S$ | 128 | 67 | 331 | 21.14 | 19.08 |
| C3 | $C_{14}H_{15}N_5O_2S$ | 126 | 70 | 331 | 18.54 | 15.87 |
| C4 | $C_{14}H_{14}N_4O_2S$ | 122 | 62 | 302 | 25.54 | 22.20 |
| C5 | $C_{12}H_{11}N_5OS$ | 130 | 68 | 274 | 16.37 | 15.68 |

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**Table 2: Infra Red / $^1$H NHR Spectral Study of the Synthesized Compounds.**

| Compound | IR ($cm^{-1}$), | H – NMR (δ, ppm) |
|---|---|---|
| **C** | 3391 (N-H str.), 1400 (Ar.C-H str.), 1628 (Ar.C-C str.), 1450 (C-N str.), 1055 (C-S str.), 1450 (C=C str.) | 7.8-8.20 (8H, Ar-CH), 6.87 (1H, NH), 4.72 (1H, NH), 4.50 (2H, $CH_2$) |
| **C1** | 3410 (N-H str.), 1460 (Ar.C-H str.), 1680 (Ar.C-C str.), 1465 (C-N str.), 1125 (C-S str.), 1550 (C=C str.), 1555 (N-O str.), 1720 (C=O str.), 1650 (N-H bend) | 10.85 (1H, OH), 6.90-8.06 (8H, Ar-CH), 4.85 (2H, $CH_2$), 6.50 (1H, NH) |
| **C2** | 3450 (N-H str.), 1475 (Ar.C-H str.), 1605 (Ar.C-C str.), 1420 (C-N str.), 1065 (C-S str.), 1560 (C=C str.) 1565 (N-O asy. str.), 1670 (N-H bend) | 10.05 (1H, OH), 6.80-8.20 (8H, Ar-CH), 4.65 (2H, $CH_2$), 6.40 (1H, NH) |
| **C3** | 3310 (N-H str.), 1460 (Ar.C-H str.), 1680 (Ar.C-C str.), 1450 (C-N str.), 1567 (N-O str.), 1020 (C-S str.), 1580 (C=C str.), 1635 (-$NH_2$ str.), 1660 (N-H bend) | 10.05 (1H, OH), 6.80-8.20 (8H, Ar-CH), 4.65 (2H, $CH_2$), 6.40 (1H, NH) |
| **C4** | 3550 (N-H str.), 1550 (Ar.C-H str.), 1690 (Ar.C-C str.), 1450 (C-N str.), 1567 (N-O str.), 1010 (C-S str.), 1500 (C=C str.), 750 (NH str.), 3455 (-OH str.), 1650 (N-H bend) | 10.85 (1H, OH), 6.50-8.25 (8H, Ar-CH), 4.85 (2H, $CH_2$), 6.70 (1H, NH) |
| **C5** | 3550 (N-H str.), 1550 (Ar.C-H str.), 1690 (Ar.C-C str.), 1010 (C-S str.), 1500 (C=C str.) 1250 (C-N str.), 750 (NH str.), 3455 (-OH str.), 1690 (N-H bend) | 10.05 (1H, OH), 6.50-8.25 (8H, Ar-CH), 4.65 (2H, $CH_2$), 6.40 (1H, NH) |

## Antibacterial Activity

All the newly synthesized 1, 3, 4-thiadiazole derivatives were screened for their antibacterial and antifungal activity. For antibacterial studies microorganisms employed were *Staphylococcus aureus* ATCC 9144*, Escherichia coli ATCC 25922,* For antifungal, *Aspergillus niger ATCC 9029 and Aspergillus fumigatus ATCC 46645* were used as organism. Both microbial studies were assessed by Minimum Inhibitory Concentration (MIC) by serial dilution method. For this, the compound whose MIC has to be determined is dissolved in serially diluted DMF Then a standard drop of the culture prepared for the assay is added to each of the dilutions, and incubated for 16–18 hrs at $37^0$c. MIC is the highest dilution of the compound, which shows clear fluid with no development of turbidity. The results are shown in the table: 3.

**Table: 3. Antibacterial and Antifungal Data for the Newly Synthesized Thiadiazoles.**

| Compd. | Antibacterial data in MIC(µg/ml) | | Antifungal data in MIC (µg/ml) | |
|---|---|---|---|---|
| | ***S. aureus*** | ***E.coli*** | ***A.niger*** | ***A.fumigatus*** |
| C1 | 9 | 8 | 18 | 19 |
| C2 | 9 | 9 | 19 | 18 |
| C3 | 4 | 5 | 14 | 16 |
| C4 | 8 | 7 | 19 | 20 |
| C5 | 6 | 5 | 15 | 16 |
| Streptomycin | 10 | 10 | -- | -- |
| Fluconazole | -- | | 20 | 22 |

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## RESULT AND DISCUSSION

The structures of the synthesized compound were determined on the basis of their FTIR and $^{1}$H NMR data. The spectral data for FTIR and 1 H NMR are elaborated in table-2, which confirms the structure of synthesized compounds. Thus, it is obvious from the structure-activity profile of substituted 1,3,4-Thiadiazole derivatives; a small structural variation may induce an effect on antibacterial activity.

## CONCLUSION

A series of novel 1,3,4-Thiadiazole derivatives were synthesized. *In vitro* antibacterial activity data of 1,3,4-Thiadiazole derivatives against tested organisms displayed significant activity with a wide degree of variation. It is found that compound **C1, C4** have shown significant antibacterial activity. Rest of the compounds has exhibited significant to substantial activity against the same strain. Substantial activity is achieved in case of compounds **C4** against *S. aureu sand E.coli,* and the remaining compounds are significantly active against the same species. All the 1,3,4-Thiadiazole derivatives have exhibited significant to moderate activity against gram positive and gram negative bacteria. While derivatives **C1, C2,** and **C4** have shown higher activity against *A. fumigates.* Comparatively weak to moderate activity has been reported by **C3** and **C5**. From *in vitro* antifungal activity (Table 3), data reveals that all the newly synthesized compounds displayed higher to week activity in comparison to standards. The structures of the entire compounds were confirmed by recording by their$^{1}$H NMR, and IR spectra. In conclusion, we feel that the preliminary *in vitro* activity results of this class of compounds may possess potential for design of future molecules with modifications. All the synthesized compounds showed higher to moderate activity against microorganism. Therefore, it is concluded that there exists ample scope for further study in this class of compounds.

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