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# A Facile, Rapid, one-pot Synthesis and Biological Evaluation of some Thiadiazole Derivatives

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## Abstract

*Some novel thiadiazole derivatives were synthesized through two routes viz.(i) single step reaction of substituted aromatic acid with thiosemicarbazide in presence of $POCl_3$ and (ii) via multistep synthesis by converting substituted aromatic acid to their corresponding esters and then into their hydrazides, which are converted into derivative of their benzoyl thiosemicarbazide followed by cyclization. The structure of the synthesized compounds has been established on the basis of IR, $^1$H-NMR and $^{13}$C-NMR and mass spectrometry. The compounds have been evaluated for antibacterial activity against Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli, Xanthomonas citrii and antifungal against Aspergillus flavus, Aspergillus niger, Alternaria solanii, Fusarium oxysporum, Colletotrichum falcatum and showed moderate to good activities.*

**Keywords:** Thiadiazole, Antibacterial activity, Antifungal activity.

## Introduction

In the last few decades, it has been observed that the numbers of new infectious disease caused by bacteria, fungi and viruses are damaging public health worldwide and multi-drug-resistant pathogens are responsible for the death of millions of patients every year. Some bacteria that are capable of causing serious disease are becoming resistant to most of commonly available antibiotics. These bacteria can spread from person to person in the community or from patient to patient in hospital and is a serious public health problem.

The treatment of fungal infections by drug-resistant fungal pathogens often creates a problem of high toxicity, low tolerability or narrow spectrum of activity. Search for potent and new antimicrobial agent to treat the resistant pathogens is one of the most challenging tasks for medicinal chemistry. All these facts demands for development of potent antimicrobial agents with unique mode of action.

During literature survey, it is found that several five member aromatic systems having three hetero atoms at symmetrical position 1,3,4 have showed interesting physiological properties. 1,3,4-thiadiazole derivatives for their diverse biological activities as antimicrobial[1-6], anti-tubercula[7-9], anti-inflammatory[10-14], anticonvulsant[15-18], antifungal[19-22], antibacterial[23], antihypertensive[24-27], antioxidant[28,29], anticancer[30-34], antidiabetic[35,36], anti-leishmanial[37-39], has become an important molecule for the development of new drugs.

Number of clinically effective drugs with 1,3,4-thiadiazole moiety are available in the market such as Megazole as antitumor, Azetepa as anticancer, Methazolamide as diuretic, Cefazolin as antibiotic, Cefozopran as antibiotic, Sulphamethizole as antibacterial etc. (Figure-1) but all these drugs are having some sever side effect.

Keeping these facts in consideration, a system combining bio-labile 1,3,4-thiadiazoles moiety and their derivatives having aromatic substituents has been designed and synthesised with the view to develop new antimicrobial drugs to treat resistant pathogens with low or no side effect.

## Material and Methods

All reagents were obtained commercially and were of the highest commercial quality and used without further purification. Solvents were freshly distilled and used. Melting points were determined using an electro thermal digital apparatus and are uncorrected. Purity of the compound was checked by thin layer chromatography (TLC).

All the starting materials were synthesized by the method given in literature and identified by $^1$HNMR spectra, IR spectra and micro analysis of these compounds were in satisfactory agreement with the structures.

IR spectra were prepared on a FT-IR spectrophotometer using KBr discs. $^1$H and $^{13}$C NMR spectra were recorded on Bruker spectrophotometer (300 MHz) in DMSO-*d6* using TMS as an internal standard. ESMS were recorded on Waters UPLC-TQD Triple Quadrupole Mass Spectrometer.

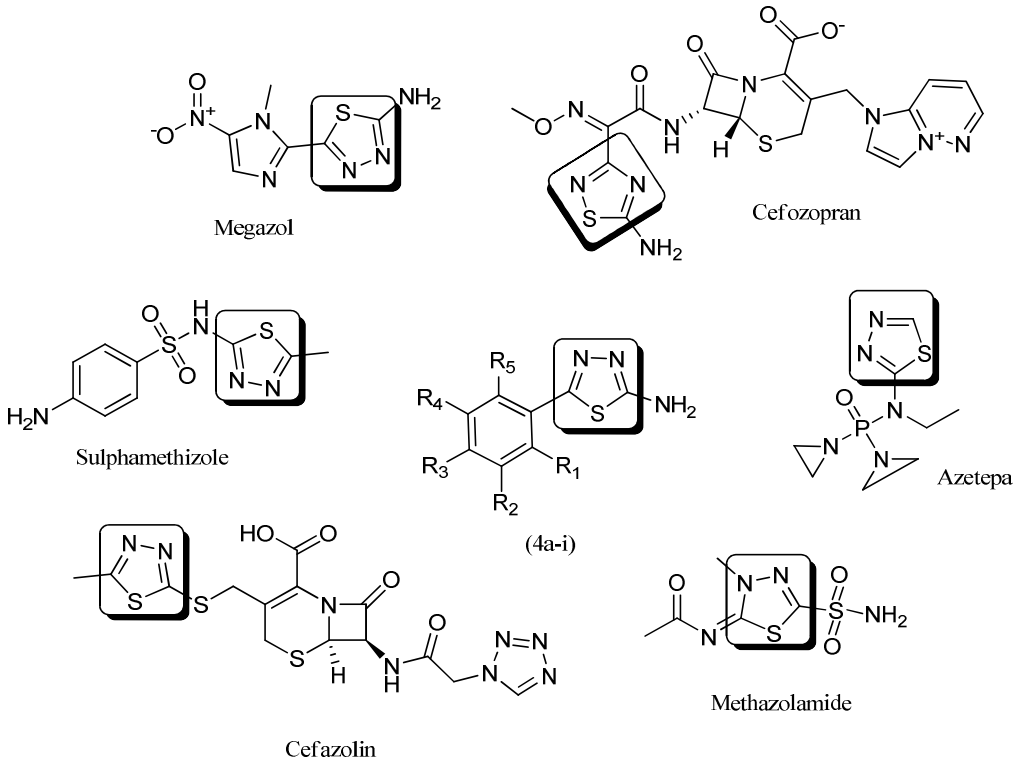



**Figure-1**
**Clinically used drugs and synthesized compounds 4a-i containing thiadiazole nucleus**

**General Procedure: Butyl substituted benzoate 1a-i:** Substituted benzoic acid (0.07 mole) in 120 mL of n-butanol and 8 ml conc. sulfuric acid was refluxed for 6 hours by using Dean-Stark apparatus, reaction mixture was cooled and treated with saturated solution of sodium carbonate followed by water and organic layer was separated. Excess butanol was distilled by vacuum distillation to get the desired product.

**Substituted benzohydrazides 2a-i:** A mixture of butyl substituted benzoate 20 ml and hydrazine hydrate (0.2 mole) in 70 ml methanol was refluxed on water bath for 9 hours. The product was isolated, filtered and washed with diethyl ether under stirring. The mass was filtered and dried followed by crystallization with ethanol to get the desire product.

**2-Aroyl hydrazinecarbothioamide 3a-i:** A mixture of substituted benzohydrazide (0.03mole), ammonium thiocyanate (0.09 mole) and n-butanol (60 mL) as a solvent was refluxed for 22 hours. Excess butanol was distilled and solid obtained was recrystallized with methanol. The physical and elemental data of different title compound **3** synthesized are recorded in Table-3.

**2-(4-methylbenzoyl) hydrazinecarbothioamide (3a)**: $^{1}$H-NMR ($CDCl_3$, 300 MHz): δ 7.49–7.89 (d, 4H, ArH), 2.31 (s, 3H, $CH_3$) 8.59 (s, 2H, $NH_2$), 7.91 (-CONH), 2.1 (CSNH); $^{13}$C-NMR ($CDCl_3$, 75 MHz): δ 180.6 (C=S), 165.9 (C=O), 142.0, 141.5, 128.6, 127.1 (aromatic carbon), 21.6 (Ar-$CH_3$); IR (KBr, $cm^{-1}$): 1300 (C=S), 1710 (C=O); ESMS: m/z 209.

**2-(4-methyl-3-nitrobenzoyl)hydrazinecarbothioamide (3b)**: $^{1}$H-NMR ($CDCl_3$, 300 MHz): δ 7.60–8.31 (d, 2H, ArH), 8.43 (s, 1H), 2.31 (s, 3H, $CH_3$) 8.55 (s, 2H, NH2), 7.91 (-CONH), 2.1 (CSNH); $^{13}$C-NMR ($CDCl_3$, 75 MHz): δ 182.6 (C=S), 164.5 (C=O), 149.0, 138.5, 133.6, 131.9, 130.2, 121.0 (aromatic carbon), 18.5 (Ar-$CH_3$); IR (KBr, $cm^{-1}$): 1330 (C=S), 1715 (C=O); ESMS: m/z 254.6.

**2-(4-methyl-3,5-dinitrobenzoyl) hydrazinecarbothioamide (3c)**: $^{1}$H-NMR ($CDCl_3$, 300 MHz): δ 8.82 (s, 1H, ArH), 8.82 (s, 1H, ArH), 2.31 (s, 3H, $CH_3$) 8.55 (s, 2H, NH2), 7.91(-CONH), 2.1 (CSNH); $^{13}$C-NMR ($CDCl_3$, 75 MHz): δ 182.6 (C=S), 164.9 (C=O), 150.0, 132.5, 130.2, 127.1 (aromatic carbon), 15.3 (Ar-$CH_3$); IR (KBr, $cm^{-1}$): 1340 (C=S), 1730 (C=O); ESMS: m/z 299.3.

**2-(3-methylbenzoyl) hydrazinecarbothioamide (3d)**: $^{1}$H-NMR ($CDCl_3$, 300 MHz): δ 7.60 (s, 1H, ArH), 7.42-7.70 (d, 2H, ArH), 7.29 (s, 1H, ArH) 2.35 (s, 3H, $CH_3$) 8.55 (s, 2H, $NH_2$), 7.91 (-CONH), 2.1 (CSNH); $^{13}$C-NMR ($CDCl_3$, 75 MHz): δ 182.6 (C=S), 164.0 (C=O), 139.5, 136.0, 132.0, 129.2, 128.0, 125.5 (aromatic carbon), 21.6 (Ar-$CH_3$); IR (KBr, $cm^{-1}$): 1340 (C=S), 1730 (C=O); ESMS: m/z 209.2.

**2-(2-methylbenzoyl) hydrazinecarbothioamide (3e)**: $^{1}$H-NMR ($CDCl_3$, 300 MHz): δ 7.38-7.65 (m, 2H, ArH), 7.32-7.80 (d, 2H, ArH), 2.35 (s, 3H, $CH_3$) 8.55 (s, 2H, $NH_2$), 7.91(-CONH), 2.1 (CSNH); $^{13}$C-NMR ($CDCl_3$, 75 MHz): δ 182.6 (C=S), 164.0

(C=O), 132.0, 130.0, 129.0, 129.5, 128.0, 127.1 (aromatic carbon), 18.6 (Ar-$CH_3$); IR (KBr, $cm^{-1}$): 1340 (C=S), 1730 (C=O); ESMS: m/z 209.01.

**2-(3-bromo-4-methylbenzoyl) hydrazinecarbothioamide (3f)**: $^1$H-NMR ($CDCl_3$, 300 MHz): δ 7.9 (s, 1H, ArH), 7.36- 7.82 (d, 2H, ArH), 2.31 (s, 3H, $CH_3$) 8.55 (s, 2H, $NH_2$), 7.91(-CONH), 2.1 (CSNH); $^{13}$C-NMR ($CDCl_3$, 75 MHz): δ 182.6 (C=S), 164.9 (C=O), 142.0, 133.5, 130.2, 129.1, 126.9, 122.3 (aromatic carbon), 25.3 (Ar-$CH_3$); IR (KBr, $cm^{-1}$):1340 (C=S), 1730 (C=O); ESMS: m/z 288.03.

**2-(3-methyl-2-nitrobenzoyl) hydrazinecarbothioamide (3g)**: $^1$H-NMR ($CDCl_3$, 300 MHz): δ 7.83 (s, 1H, ArH), 8.82 (s, 1H, ArH), 2.31 (s, 3H, $CH_3$) 8.55 (s, 2H, $NH_2$), 7.91 (-CONH), 2.1 (CSNH); $^{13}$C-NMR ($CDCl_3$, 75 MHz): δ 182.6 (C=S), 164.9 (C=O), 150.1, 139.5, 136.2, 132.6, 125.1, 123.7 (aromatic carbon), 18.6 (Ar-$CH_3$); IR (KBr, $cm^{-1}$): 1340 (C=S), 1730 (C=O); ESMS: m/z 254.3.

**2-(5-methyl-2,4-dinitrobenzoyl) hydrazinecarbothioamide (3h)**: $^1$H-NMR ($CDCl_3$, 300 MHz): δ 8.72 (s, 1H, ArH), 8.12 (s, 1H, ArH), 2.51 (s, 3H, $CH_3$) 8.55 (s, 2H, $NH_2$), 7.91(-CONH), 2.1 (CSNH); $^{13}$C-NMR ($CDCl_3$, 75 MHz): δ 182.6 (C=S), 164.9 (C=O), 152.0, 142.5, 136.2, 128.1, 127.6, 121.5 (aromatic carbon), 18.6 (Ar-$CH_3$); IR (KBr, $cm^{-1}$): 1340 (C=S), 1730 (C=O); ESMS: m/z 299.

**2-(2-methyl-3,5-dinitrobenzoyl) hydrazinecarbothioamide (3i)**: $^1$H-NMR ($CDCl_3$, 300 MHz): δ 8.82 (s, 1H, ArH), 9.02 (s, 1H, ArH), 2.31 (s, 3H, $CH_3$) 8.55 (s, 2H, $NH_2$), 7.91(-CONH), 2.1 (CSNH); $^{13}$C-NMR ($CDCl_3$, 75 MHz): δ 182.6 (C=S), 164.9 (C=O), 149.5, 146.5, 136.2, 132.1, 129.5, 122.1 (aromatic carbon), 14.8 (Ar-$CH_3$). IR (KBr, $cm^{-1}$): 1340 (C=S), 1730 (C=O); ESMS: m/z 299.5.

**5-Aryl-1,3,4-thiadiazole-2-amine 4a-i:** Conc. Sulphuric acid (25mL) was added dropwise to 2-Aroyl hydrazinecarbothioamide (0.02 mole) at 0-10$^0$C under stirring. Reaction was allowed to stand for half an hour at 0-10$^0$C then maintained at 25-30$^0$C for 1 hour under stirring. The reaction mass was poured into crushed ice, the solid precipitated was filtered and washed with water. The obtained mass was recrystallized with ethanol.

**One step synthesis of 5-Aryl-1,3,4-thiadiazole-2-amine 4a-i:** A mixture of substituted benzoic acid (0.007 mole) and thiosemicarbazide (0.006 mole) was taken and kept in ice bath at 5-10$^0$C under stirring then added phosphorous oxychloride (0.027 mole) dropwise. The reaction mixture was heated to 75-80$^0$C for 1 hour. After cooling 7.5mL distilled water was added to reaction mixture and refluxed for 4-5 hours. Reaction mixture was cooled upto 25-30$^0$C and neutralized with potassium hydroxide. Precipitate was filtered, washed with water and recrystallized with ethanol. The physical and elemental data of different title compound 4 synthesized are recorded in Table-4.

**5-(4-methylphenyl)-1,3,4-thiadiazole-2-amine (4a)**: $^1$H-NMR ($CDCl_3$, 300 MHz): δ 7.81–8.19 (d, 4H, ArH), 2.31 (s, 3H, $CH_3$) 6.95 (s, 2H, $NH_2$); $^{13}$C-NMR ($CDCl_3$, 75 MHz): δ 174.2 (C-5), 160.3 ($H_2$N-C-2), 131.3, 130.1, 129.7, 126.1, (aromatic carbon), 21.5 (Ar-$CH_3$). IR (KBr, $cm^{-1}$): 1030.65 (C-S-C), 3396.23 ($NH_2$). 1611.72 (C=N); ESMS: m/z 191.7.

**5-(4-methyl-3-nitrophenyl)-1,3,4-thiadiazole-2-amine (4b)**: $^1$H-NMR ($CDCl_3$, 300 MHz): δ 7.57–8.28(d, 2H, ArH), 8.28 (s, 1H, ArH) 2.31 (s, 3H, $CH_3$) 6.95 (s, 2H, $NH_2$); $^{13}$C-NMR ($CDCl_3$, 75 MHz): δ 174.0 (C-5), 158.5 ($H_2$N-C-2), 148.6, 133.3, 132.8, 131.5, 130.1, 121.8 (aromatic carbon), 18.9 (Ar-$CH_3$). IR (KBr, $cm^{-1}$): 1068.73 (C-S-C), 3423.56 ($NH_2$), 1628.2 (C=N); ESMS: m/z 236.8.

**5-(4-methyl-3, 5-dinitrophenyl)-1, 3, 4-thiadiazole-2-amine (4c)**: $^1$H-NMR ($CDCl_3$, 300 MHz): δ 7.73-8.52(s, 2H, ArH), 2.46 (s, 3H, $CH_3$) 6.95 (s, 2H, $NH_2$); $^{13}$C-NMR ($CDCl_3$, 75 MHz): δ 173.9 (C-5), 159.7 ($H_2$N-C-2), 150.3, 131.7, 128.2, 126.9 (aromatic carbon), 15.4 (Ar-$CH_3$); IR (KBr, $cm^{-1}$): 1059.27 (C-S-C), 3437.42 ($NH_2$), 1617.43 (C=N); ESMS: m/z 281.9.

**5-(3-methylphenyl)-1, 3, 4-thiadiazole-2-amine (4d)**: $^1$H-NMR ($CDCl_3$, 300 MHz): δ 7.30 (m, 1H, ArH) 7.16-7.81 (d, 2H, ArH) 7.75 (s, 1H, ArH) 2.31 (s, 3H, $CH_3$) 6.95 (s, 2H, $NH_2$); $^{13}$C-NMR ($CDCl_3$, 75 MHz): δ 174.0 (C-5), 161.5 ($H_2$N-C-2), 138.6, 132.9 130.7, 129.0, 128.5, 127.5 (aromatic carbon), 21.3 (Ar-$CH_3$). IR (KBr, $cm^{-1}$): 1030.27 (C-S-C), 3398.56 ($NH_2$), 1612.43 (C=N); ESMS: m/z 191.3.

**5-(2-methylphenyl)-1, 3, 4-thiadiazole-2-amine (4e)**: $^1$H-NMR ($CDCl_3$, 300 MHz): δ 7.30 (m, 2H, ArH) 7.16-7.81 (d, 2H, ArH) 7.75 (s, 1H, ArH) 2.31 (s, 3H, $CH_3$) 6.95 (s, 2H, $NH_2$); $^{13}$C-NMR ($CDCl_3$, 75 MHz): δ 174.2 (C-5), 161.9 ($H_2$N-C-2), 138.6, 137.1 130.5, 129.2, 128.0, 126.9 (aromatic carbon), 19.3 (Ar-$CH_3$). IR (KBr, $cm^{-1}$): 1030.25 (C-S-C), 3389.21 ($NH_2$). 1611.27 (C=N); ESMS: m/z 191.6.

**5-(3-bromo-4-methylphenyl)-1,3,4-thiadiazol-2-amine (4f)**: $^1$H-NMR ($CDCl_3$, 300 MHz): δ 7.30 (s, 1H, ArH) 7.0-7.59 (d, 2H, ArH), 2.31 (s, 3H, CH3) 6.95 (s, 2H, $NH_2$); $^{13}$C-NMR ($CDCl_3$, 75 MHz): δ 174.0 (C-5), 161.2 ($H_2$N-C-2), 138.6, 133.3 133.0, 132.1, 127.2, 125.3 (aromatic carbon), 23.4 (Ar-$CH_3$). IR (KBr, $cm^{-1}$): 1051.58 (C-S-C), 3426.31 ($NH_2$). 1614.23 (C=N); ESMS: m/z 270.4.

**5-(3-methyl-4-nitrophenyl)-1,3,4-thiadiazol-2-amine (4g)**: $^1$H-NMR ($CDCl_3$, 300 MHz): δ 7.81-8.19 (d, 2H, ArH), 8.0(s, 1H, ArH),2.31 (s,3H,$CH_3$) 6.95 (s, 2H, $NH_2$); $^{13}$C-NMR ($CDCl_3$, 75 MHz): δ 174.3 (C-5), 160.5 ($H_2$N-C-2), 138.9, 136.3, 133.3, 133.0, 131.9, 125.7 (aromatic carbon), 19.1 (Ar-$CH_3$); IR (KBr, $cm^{-1}$): 1071.15 (C-S-C), 3431.23 ($NH_2$). 1625.56 (C=N); ESMS: m/z 236.8.

**5-(5-methyl-2, 4-dinitrophenyl) -1,3,4-thiadiazol-2-amine (4h)**: $^1$H-NMR ($CDCl_3$, 300 MHz): δ 7.81–8.19 (d, 2H, ArH), 8.0(s, 1H, ArH), 2.31 (S, 3H, $CH_3$) 6.95 (s, 2H, $NH_2$); $^{13}$C-NMR ($CDCl_3$, 75 MHz): δ 174.3 (C-5), 161.1 ($H_2N$-C-2), 150.7, 149.1, 139.2, 138.3, 132.9, 121.0 (aromatic carbon), 18.9 (Ar-$CH_3$); IR (KBr, $cm^{-1}$): 1065.21 (C-S-C), 3446.14 ($NH_2$). 1618.72 (C=N); ESMS: m/z 281.8.

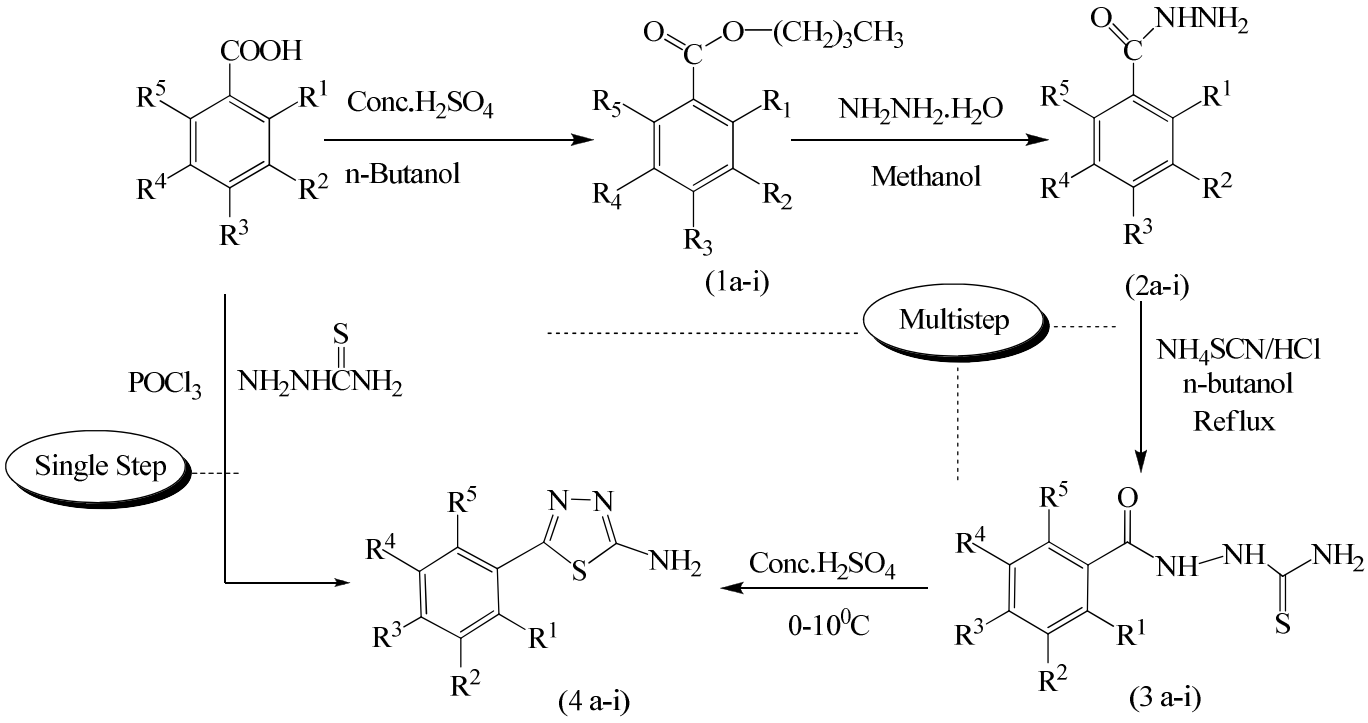
**5-(2-methyl-3,5-dinitrophenyl)-1,3,4-thiadiazol-2-amine (4i)**: $^1$H-NMR ($CDCl_3$, 300 MHz): δ 8.79 (s, 1H, ArH), 8.90(s, 1H, ArH),2.31 (S,3H,$CH_3$) 6.95 (s, 2H, $NH_2$); $^{13}$C-NMR ($CDCl_3$, 75 MHz): δ 174.0 (C-5),161.3 ($H_2N$-C-2), 151.0,146.3,139.3, 137.8,129.3, 117.3 (aromatic carbon), 14.7 (Ar-$CH_3$); IR (KBr, $cm^{-1}$): 1068.23 (C-S-C), 3449.32 ($NH_2$). 1619.42 (C=N); ESMS: m/z 281.5.

## Results and Discussion

The synthesis of 5-aryl-1,3,4-thiadiazole-2-amines 4a-i was achieved with a versatile and efficient synthetic route outlined in Scheme-1. The one-pot reaction between substituted benzoic acid, thiosemicarbazide and $POCl_3$ in water at $100^0$C resulted 4a–i. This compound was synthesizes alternatively via the formation of intermediates viz. esters 1, which on reaction with hydrazine hydrate afforded hydrazides 2, this further on refluxing with $NH_4SCN$ and HCl in n-butanol gave substituted thiosemicarbazides 3a-i, which on cyclodehydration with Conc. sulfuric acid in cold condition resulted compounds 4a-i in quantitative yield. The yield of compounds 4a-i obtained from single step process is better as compare to multistep process. The comparative yields are reported in Table-4.

The authenticity of this speculation was confirmed by an experiment in which the intermediates were isolated, characterized and then subjected to cyclodehydration reaction in cold condition to give 4a–i. super imposable IR, $^1$H-NMR, $^{13}$C-NMR spectra as well as mixed melting points and elemental analysis confirmed the authenticity of the final products formed by using one-step and multi-step methods.

The IR data of the compound 3b showed band at 1715 $cm^{-1}$ due to carbonyl group and at 1330 $cm^{-1}$ due to C=S. In IR spectra of compound 4b appearance of a new peak at 1068 $cm^{-1}$ due to C-S-C, 1628 $cm^{-1}$ due to C=N and disappearance of peaks at 1715 $cm^{-1}$ (C=O) and 1330 $cm^{-1}$ (C=S) clearly showed cyclodehydration reaction to give compound 4b, a strong stretching band is also around 3423 $cm^{-1}$ due to N-H.




a) $R^1$= H, $R^2$= H, $R^3$= $CH_3$, $R^4$= H, $R^5$= H
b) $R^1$= H, $R^2$= H, $R^3$= $CH_3$, $R^4$= $NO_2$, $R^5$= H
c) $R^1$= H, $R^2$= $NO_2$, $R^3$= $CH_3$, $R^4$= $NO_2$, $R^5$= H
d) $R^1$= H, $R^2$= $CH_3$, $R^3$= H, $R^4$= H, $R^5$= H
e) $R^1$= $CH_3$, $R^2$= H, $R^3$= H, $R^4$= H, $R^5$= H
f) $R^1$= H, $R^2$= Br, $R^3$= $CH_3$, $R^4$= H, $R^5$= H
g) $R^1$= $NO_2$, $R^2$= $CH_3$, $R^3$= H, $R^4$= H, $R^5$= H
h) $R^1$=H, $R^2$= $CH_3$, $R^3$= $NO_2$, $R^4$= H, $R^5$= $NO_2$
i) $R^1$= $CH_3$, $R^2$= $NO_2$, $R^3$= H, $R^4$= $NO_2$, $R^5$= H

**Scheme-1**
**Synthetic routes of novel thiadiazoles**

The $^1$H-NMR also confirms the structures of compounds. In the $^1$H-NMR spectrum of compound 3b, peak at δ 2.1 ppm due to thioamide proton (-CSNH), singlet at δ 2.3 ppm due to methyl protons(-$CH_3$), singlet at δ 8.5 ppm due to $NH_2$, δ 7.9 ppm due to amide proton and aromatic protons at δ 7.4-7.8 ppm. In $^1$H-NMR spectrum of compound 4b disappearance of thioamide proton, amide proton and appearance of $NH_2$ at 6.9 δ ppm clearly indicate the formation of titled compounds. $^{13}$C-NMR of 3b showed 9 peaks, tiled compound 4b evidenced the carbon skeleton with 9 peaks of newly synthesized thiadiazoles at δ 158.5 ppm, and δ 148.6 ppm due to imine carbons, δ 18.5 ppm due to $CH_3$ carbon on phenyl ring.

**Evaluation of antimicrobial activity:** The thiadiazoles 4a-i were tested for their antibacterial and antifungal in vitro activity against three strains of Gram-negative bacteria, two strains of Gram-positive bacteria, and five strains of pathogenic fungi. Respective minimum inhibitory concentration (MIC) value was determined by a disc-diffusion method[40]. The comparative activities of the newly synthesized compounds 4a-i and the standard drug Ampicillin and Fluconazole on bacterial and fungal strains respectively, are summarized in Table-1 and Table-2.

**Antibacterial activity:** The antibacterial screening (Figure-2) revealed that, all the tested compounds showed good inhibition against various tested microbial strains compared to the standard drug. Among the synthesized compounds 4e is found to be most active whereas 4d and 4g are moderate active against *Pseudomonas aureginosa* as compared to the standard. Compound 4a and 4e are more active whereas 4d, 4f and 4i are moderate active against *Staphylococcus aureus* as compared to standard. Compound 4i is most active whereas 4d, 4g and 4e are moderate active against *Escherichia coli* as compared to standard. Compound 4d, 4e, 4h and 4i are more active whereas 4a and 4f are moderate active against *Xanthomonas citrii* as compared to standard. Compound 4a, 4d, 4g, 4h and 4i are more active whereas 4e and 4f are moderate active against *Bacillus subtilis* as compared to standard. The above data showed that the enhanced antibacterial activity of the compounds may either be due to presence of nitro and methyl group in phenyl ring at different position or due to combined effect of both amino thiadiazole and phenyl core rings. Because of presence of active functional groups on both the rings (amino, nitro and methyl), SAR could be established in this series of compounds.

**Evaluation of antifungal activity:** The synthesized compounds were also evaluated against pathogenic fungal strains (Figure-3) and Fluconazole was taken as a standard drug throughout the experiment. Compound 4d is found most active against *Aspergillus niger* as compare to standard Fluconazole. The compound 4e and 4i are more active against *Alternaria solanii* as compare to standard. The compound 4g and 4i are found to exhibit antifungal activity with equal potentiality against *Aspergillus flavus* whereas the compound 4h and 4i are found equal potentiality against *Colletotrichum falcatum* as compare to standard with MIC 0.15mg/mL. The compound 4g is found to exhibit equal potentiality against *Fusarium oxysporum* as standard drug Fluconazole with MIC 0.15mg/mL. The reason might either be due to presence of nitro and methyl group in phenyl ring at different position or due to combined effect of both amino thiadiazole and phenyl core rings. The rest compounds containing different substitution on phenyl ring showed moderate activity.

**Table-1**
**Antibacterial activity of the compounds 4a-i**

| **Minimum inhibitory concentrations (MICs) of compounds** | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Compd** | **R1** | **R2** | **R3** | **R4** | **R5** | **MICs (mg/mL)** | | | | |
| | | | | | | ***P. auriginosa*** | ***S. aureus*** | ***E. coli*** | ***X. citrii*** | ***B. subtilis*** |
| 4a | H | H | $CH_3$ | H | H | 0.62 | 0.15 | 0.62 | 0.31 | 0.15 |
| 4b | H | H | $CH_3$ | $NO_2$ | H | --- | --- | --- | --- | --- |
| 4c | H | $NO_2$ | $CH_3$ | $NO_2$ | H | --- | --- | --- | --- | --- |
| 4d | H | $CH_3$ | H | H | H | 0.31 | 0.31 | 0.31 | 0.15 | 0.15 |
| 4e | $CH_3$ | H | H | H | H | 0.15 | 0.15 | 0.31 | 0.15 | 0.31 |
| 4f | H | Br | $CH_3$ | H | H | 0.62 | 0.31 | 0.62 | 0.31 | 0.31 |
| 4g | $NO_2$ | $CH_3$ | H | H | H | 0.31 | 1.25 | 0.31 | 0.62 | 0.15 |
| 4h | H | $CH_3$ | $NO_2$ | H | $NO_2$ | 1.25 | 1.25 | 0.62 | 0.15 | 0.15 |
| 4i | $CH_3$ | $NO_2$ | H | $NO_2$ | H | 0.62 | 0.31 | 0.15 | 0.15 | 0.15 |
| Ampicillin | | | | | | 1.25 | 5.0 | 2.5 | 2.5 | 1.25 |

--- = No activity found

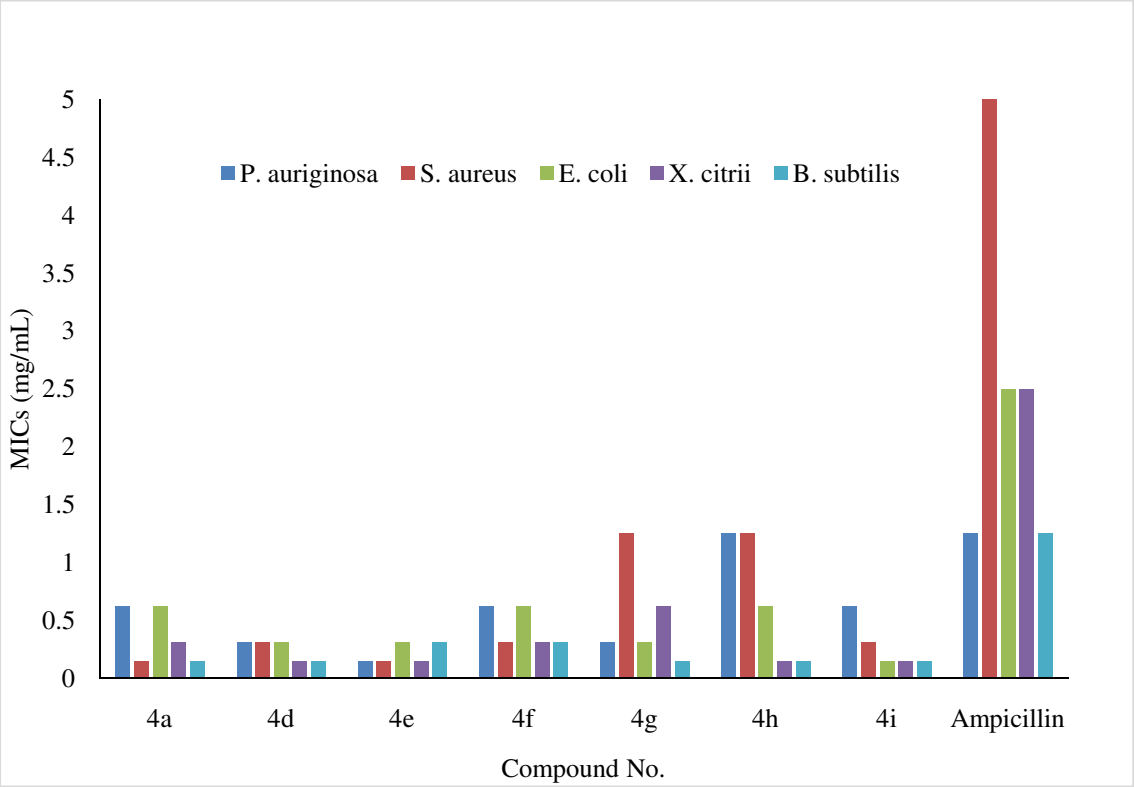



**Figure-2**
**Antibacterial activity of the compounds 4a-i**

**Table-2**
**Antifungal activity of the compounds 4a-i**

| **Minimum inhibitory concentrations (MICs) of compounds** | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Compd** | **R1** | **R2** | **R3** | **R4** | **R5** | **MICs (mg/mL)** | | | | |
| | | | | | | ***A. flavus*** | ***A. niger*** | ***F. oxysporum*** | ***A. solanii*** | ***C. falcatum*** |
| 4a | H | H | $CH_3$ | H | H | 0.31 | 0.62 | 0.62 | 0.31 | 0.62 |
| 4b | H | H | $CH_3$ | $NO_2$ | H | 1.25 | 0.62 | 0.62 | 0.31 | 0.62 |
| 4c | H | $NO_2$ | $CH_3$ | $NO_2$ | H | 0.31 | 1.25 | 0.62 | 0.62 | 1.25 |
| 4d | H | $CH_3$ | H | H | H | 0.62 | 0.15 | 0.31 | 0.62 | 0.31 |
| 4e | $CH_3$ | H | H | H | H | 0.62 | 0.31 | 0.62 | 0.15 | 1.25 |
| 4f | H | Br | $CH_3$ | H | H | 0.62 | 0.62 | 2.5 | 1.25 | 1.25 |
| 4g | $NO_2$ | $CH_3$ | H | H | H | 0.15 | 0.62 | 0.15 | 1.25 | 0.62 |
| 4h | H | $CH_3$ | $NO_2$ | H | $NO_2$ | 0.31 | 0.62 | 0.31 | 0.31 | 0.15 |
| 4i | $CH_3$ | $NO_2$ | H | $NO_{2s}$ | H | 0.15 | 0.62 | 0.31 | 0.15 | 0.15 |
| Fluconazole | | | | | | 0.15 | 0.31 | 0.15 | 0.31 | 0.15 |

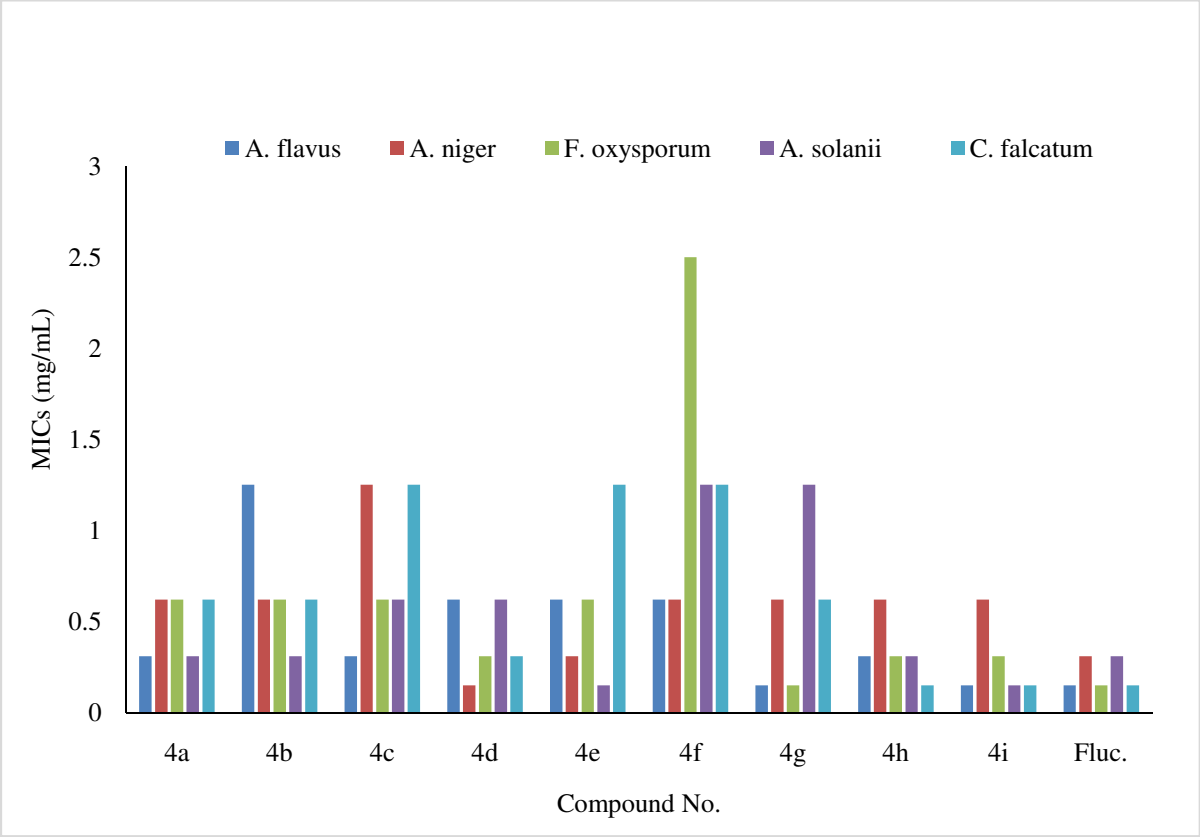



**Figure-3**
**Antifungal activity of the compounds 4a-i**

**Table-3**
**Physical and elemental data of compounds 3a-i**

| Compd | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ | Yield | m.p. | $R_f^*$ | Mol. formula | Found (Calcd) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | (%) | °C | | | C | H | N |
| 3a | H | H | $CH_3$ | H | H | 41 | 230 | 0.73 | $C_9H_{11}N_3OS$ | 51.63 (51.65) | 5.31 (5.30) | 20.04 (20.08) |
| 3b | H | H | $CH_3$ | $NO_2$ | H | 46 | 222-223 | 0.79 | $C_9H_{10}N_4O_3S$ | 42.51 (42.50) | 3.99 (3.96) | 22.10 (22.04) |
| 3c | H | $NO_2$ | $CH_3$ | $NO_2$ | H | 43 | 220 | 0.75 | $C_9H_9N_5O_5S$ | 36.10 (36.12) | 3.00 (3.03) | 23.42 (23.40) |
| 3d | H | $CH_3$ | H | H | H | 45 | 231-232 | 0.70 | $C_9H_{11}N_3OS$ | 51.63 (51.65) | 5.31 (5.30) | 20.04 (20.08) |
| 3e | $CH_3$ | H | H | H | H | 45 | 228-229 | 0.68 | $C_9H_{11}N_3OS$ | 51.63 (51.65) | 5.31 (5.30) | 20.04 (20.08) |
| 3f | H | Br | $CH_3$ | H | H | 46 | 240 | 0.63 | $C_9H_{10}BrN_3OS$ | 37.53 (37.51) | 3.53 (3.50) | 14.50 (14.58) |
| 3g | $NO_2$ | $CH_3$ | H | H | H | 61 | 242-243 | 0.69 | $C_9H_{10}N_4O_3S$ | 42.51 (42.50) | 3.99 (3.96) | 22.10 (22.04) |
| 3h | H | $CH_3$ | $NO_2$ | H | $NO_2$ | 46 | 210 | 0.71 | $C_9H_9N_5O_5S$ | 36.10 (36.12) | 3.00 (3.03) | 23.42 (23.40) |
| 3i | $CH_3$ | $NO_2$ | H | $NO_2$ | H | 43 | 235-236 | 0.77 | $C_9H_9N_5O_5S$ | 36.10 (36.12) | 3.00 (3.03) | 23.42 (23.40) |

Solvent system: Ethyl acetate-acetone (4 : 1)

**Table-4**
**Physical and elemental data of compounds 4a-i**

| Compd | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ | Yield (%) | | m.p. °C | $R_f$* | Mol. formula | Found (Calcd) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | By single step | By multi step | | | | C | H | N |
| 4a | H | H | $CH_3$ | H | H | 80 | 21 | 215 | 0.63 | $C_9H_9N_3S$ | 56.50 (56.52) | 4.69 (4.74) | 21.94 (21.97) |
| 4b | H | H | $CH_3$ | $NO_2$ | H | 90 | 15 | 205 | 0.67 | $C_9H_8N_4O_2S$ | 45.70 (45.75) | 3.40 (3.41) | 23.69 (23.72) |
| 4c | H | $NO_2$ | $CH_3$ | $NO_2$ | H | 86 | 16 | 220-221 | 0.71 | $C_9H_7N_5O_4S$ | 38.40 (38.43) | 2.49 (2.51) | 24.89 (24.90) |
| 4d | H | $CH_3$ | H | H | H | 62 | 20 | 230 | 0.65 | $C_9H_9N_3S$ | 56.50 (56.52) | 4.69 (4.74) | 21.94 (21.97) |
| 4e | $CH_3$ | H | H | H | H | 64 | 19 | 239-240 | 0.69 | $C_9H_9N_3S$ | 56.50 (56.52) | 4.69 (4.74) | 21.94 (21.97) |
| 4f | H | Br | $CH_3$ | H | H | 76 | 22 | 195-196 | 0.73 | $C_9H_8BrN_3S$ | 40.03 (40.01) | 2.95 (2.98) | 15.53 (15.55) |
| 4g | $NO_2$ | $CH_3$ | H | H | H | 65 | 17 | 160 | 0.79 | $C_9H_8N_4O_2S$ | 45.70 (45.75) | 3.38 (3.41) | 23.75 (23.72) |
| 4h | H | $CH_3$ | $NO_2$ | H | $NO_2$ | 63 | 20 | 184-85 | 0.68 | $C_9H_7N_5O_4S$ | 38.41 (38.43) | 2.55 (2.51) | 24.89 (24.90) |
| 4i | $CH_3$ | $NO_2$ | H | $NO_2$ | H | 79 | 21 | 246-247 | 0.73 | $C_9H_7N_5O_4S$ | 38.45 (38.43) | 2.50 (2.51) | 24.91 (24.90) |

Solvent system: Ethyl acetate-acetone (4 : 1)

## Conclusion

The present work led to the development of novel antimicrobial molecules containing thiadiazole pharmacophore through two routes viz. i. efficient single step in presence of $POCl_3$ and thiosemicarbazide and ii. via multistep synthesis by substituted aromatic acid followed by intramolecular cyclization of 4-methyl benzoyl thiosemicarbazides in presence of conc. Sulphuric acid. Thus, we have found an effective and simple reaction for the preparation of novel thiadiazole derivatives. These novel compounds were evaluated in vitro, for antibacterial activity against *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli, Xanthomonas citrii* and antifungal activity against *Aspergillus flavus*, *Aspergillus niger*, *Alternaria solanii, Fusarium oxysporum*, *Colletotrichum falcatum.* The compounds showed good antibacterial activity against tested fungi and moderate antifungal activity. Hence this study has widened the scope of developing these types of thiadiazole derivatives as promising antimicrobial agents.

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