Synthesis of 1,3,4-Thiadiazole-Containing Azo Dyes and its pharmacological studies

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Abstract
The 1,3,4-thiadiazole nucleus is one of the most important and well-known heterocyclic nuclei, which is a common and integral feature of a variety of natural products and medicinal agents . A series of azo dyes derived from 2-amino-5-aryl-1,3,4-thiadiazoles were synthesized in high yields by a conventional diazotization-coupling sequence. The chemical structures of the prepared compounds were confirmed by ^{1}H-NMR, ^{13}C-NMR, IR, , mass spectrometry and these molecules were screened for antimicrobial activity molecule with nitro substituents gave promising results than other derivatives.

Keywords: 1,3,4-thiadiazole scaffolds pharmacological activities

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Introduction

Thiadiazoles are uncommon five-membered heterocyclic structures in nature. In such a ring, nitrogen, sulphur, and carbon atoms can be organised in a variety of ways, giving birth to a number of isomers: 1,2,3, 1,2,4, 1,2,5, and 1,3,4 thiadiazole are examples of related compounds. [**1**,**2**]. Scientists seem to prefer the 1,3,4-thiadiazole derivatives over the other three isomers. It has been shown that several compounds with this structure exhibit a variety of biological interactions and possess antifungal [3,4], antibacterial [5,] anti-inflammatory [6,] and anticancer [7] activities. It is crucial to underline their utilisation in addition to other industrial applications as viscosity stabilisers in rubber manufacturing [8], additives for making lithium battery electrodes [9], dyes [10], and optoelectronic materials [11]. Other instances of 2,5-dimercapto-1,3,4-thiadiazoles and 1,3,4-thiadiazole derivatives being utilised as lubricants have been reported [12].

Azo dyes, which may be recognised by the presence of an azo moiety (N=N) in their structure, are the most significant class of dispersion dyes [13]. They are utilised in many different industries, primarily the manufacture of dyestuffs but also in the food, cosmetics, and pharmaceutical sectors. Generally speaking, azo dyes offer brilliant colours in a variety of hues, including yellows, oranges, reds, and blues. One of the subgroups of this family consists of conjugated 1,3,4-thiadiazoles with an azo group (N=N) in their structure. These heterocyclic azo dispersion dyes have attracted the attention of scientists due to their clarity, brightness, and affinity for a range of fibres.[**14**,**15**] A two-step transformation is most typically utilised to introduce this kind of group into a final azo product by the application of the appropriate diazonium salts [16]. When primary aromatic amines and nitrites interact at low temperatures and in the presence of strong mineral acids, the latter are frequently produced. Due to their extreme instability, diazonium salts are used straight away once they are produced in coupling reactions with phenols or amines and substituted with electron-donating groups [17]. Nitro compound condensation with amines [18], nitro compound reduction [19,20], amine oxidation [21,22], and nitroso compound condensation with amines are further methods for making azo colours. [23] Despite the fact that thiadiazoles contain a significant thiazole chromophoric core in their structure, 1,3,4-thiadiazole-2-amine derivatives are seldom described in the literature

as being diazotized and then coupled [24,25,26]. An additional electronegative nitrogen atom may have been inserted, which would have a negative effect on the outer amino group's basicity and reactivity.

Experimental Section

The melting points were determined using the Electrothermal 9100 instrument. As KBr pellets, the IR spectra were obtained using an FTIR Bruker-vector 22 spectrophotometer. The 1H and 13C NMR spectra were recorded on a Varian Gemini NMR spectrometer in CDCl3 or DMSO-d6 as a solvent at 300 MHz and 75 MHz, respectively, with TMS as an internal standard. Chemical shifts were recorded in ppm levels. Shimadzu GCMS-QP-1000 EX mass spectrometer in EI (70 eV) mode was used to collect mass spectra.

General Procedure for the synthesis of 2-(5-(Substitutedphenyl)thiophene-2-carbonyl)hydrazine-1-carbothioamide .2(a-f)

A carboxylic acid (0.10 mol) and thionyl chloride (22 mL, 0.30 mol) were refluxed in dry toluene (10 mL) until the acid was fully consumed (TLC; 5–15 h). After cooling, the mixture was concentrated on a rotary evaporator, washed with additional dry toluene (10 mL) and concentrated again. The crude acid chloride was then dissolved in 50 mL of dry toluene and added dropwise to a mixture of thiosemicarbazide (9.11 g, 0.10 mol), $NaHCO_3$ (8.40 g, 0.10 mol) and H_2O (150 mL). The whole solution was agitated at room temperature overnight. The precipitated solid was filtered off, dried in air and recrystallized from a mixture of EtOH–H_2O to obtain pure 2-benzoylhydrazinecarbothioamide derivatives.

General Procedure for the Synthesis of 5-(5-(para substituted phenyl)thiophen-2-yl)-1,3,4-thiadiazol-2-amine. 4(a-f)

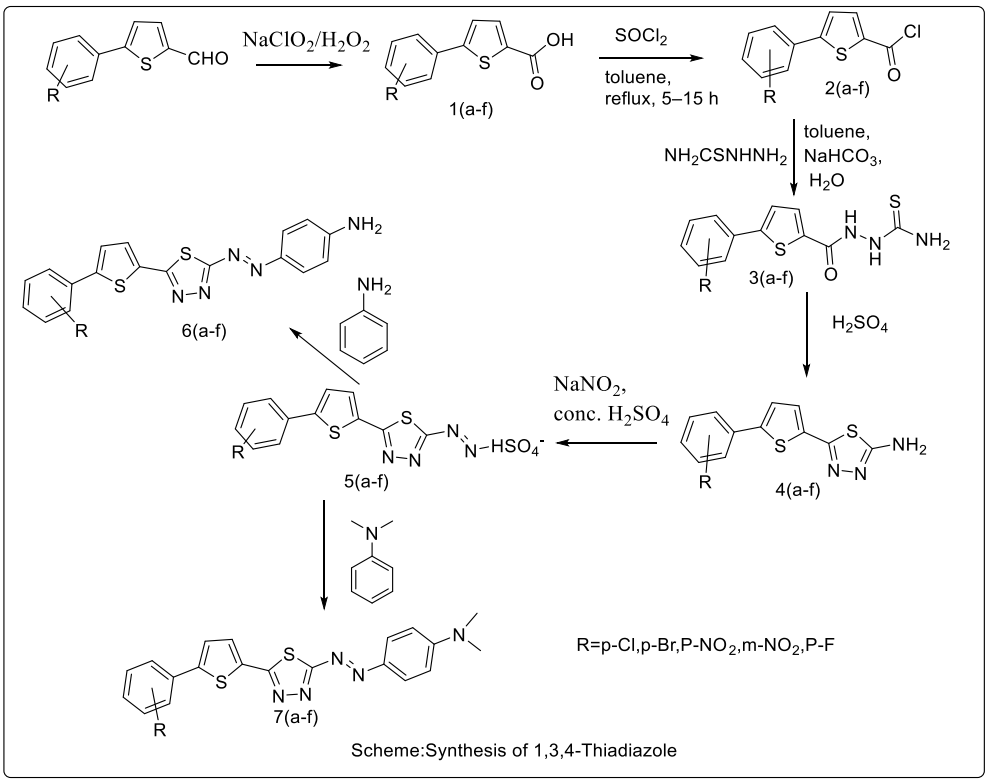
Derivatives of 2-(5-(Substitutedphenyl)thiophene-2-carbonyl)hydrazine-1-carbothioamide (2a–e) (0.05 mol) were dissolved in 50 mL of concentrated H_2SO_4 and agitated at room temperature overnight. Then, the mixture was alkalized with 25% ammonia and the precipitated product was filtered off, dried in air and recrystallized from a mixture of EtOH–H_2O to yield the corresponding 2-amino-1,3,4-thiadiazoles.

General Procedure for the Synthesis of 4-((5-(5-(para substituted phenyl) thiophen-2-yl)-1,3,4-thiadiazol-2-yl)diazenyl)aniline. 6(a-f)

Sodium nitrite (1.31 g, 0.019 mol) was introduced portion wise into concentrated sulfuric acid (20 mL). The solution was heated to 50 °C in a water bath until complete dissolution and then rapidly cooled in an ice/salt bath to 0 °C. In the meantime, the solution of the appropriate 5'-(Substituted phenyl)-[2,2'-bithiophen]-5-amine 3a–e (0.015 mol) in glacial acetic acid (30 mL) and propionic acid (15 mL) was prepared and added dropwise to an agitated solution of sodium nitrite in concentrated sulfuric acid at 0–5 °C. Then, the mixture was stirred for 24 h, and excess nitrous acid was decomposed by the addition of urea. The resulting diazonium salt solution was slowly introduced into the mixture of aniline (1.37 mL, 1.39 g, 0.015 mol) in 15 mL of water at 0–5 °C. The colored mixture was stirred at room temperature for the next 24 h and finally neutralized with saturated sodium carbonate solution (75 mL). The solid was filtered off, washed twice with hot water (2 × 25 mL) and dried in air. The crude product (4a–e) was purified by column chromatography on silica gel ($CHCl_3$/EtOAc, 5:1 v/v).

General Procedure for the Synthesis of 4-((5-(5-(4-substitutedhenyl)thiophen-2-yl)-1,3,4-thiadiazol-2-yl)diazenyl)-N,N-dimethylaniline.7(a-f)

Sodium nitrite (1.31 g, 0.019 mol) was introduced portion wise into concentrated sulfuric acid (20 mL). The solution was heated to 50 °C in a water bath until complete dissolution and then rapidly cooled in an ice/salt bath to 0 °C. In the meantime, the solution of the appropriate 5'-(Substituted phenyl)-[2,2'-bithiophen]-5-amine 3a–e (0.015 mol) in glacial acetic acid (30 mL) and propionic acid (15 mL) was prepared and added dropwise to an agitated solution of sodium nitrite in concentrated sulphuric acid at 0–5 °C. Then, the mixture was stirred for 24 h, and excess nitrous acid was decomposed by the addition of urea. The resulting diazonium salt solution was slowly introduced into the mixture of dimethyl aniline (1.37 mL, 1.39 g, 0.015 mol) in 15 mL of water at 0–5 °C. The colored mixture was stirred at room temperature for the next 24 h and finally neutralized with saturated sodium carbonate solution (75 mL). The solid was filtered off, washed twice with hot water (2 × 25 mL) and dried in air. The crude product (4a–e) was purified by column chromatography on silica gel ($CHCl_3$/EtOAc, 5:1 v/v).



Scheme:Synthesis of 1,3,4-Thiadiazole

Spectral data of Synthesised Compounds

Compound 1a

^{1}H NMR: δ 6.88 (2H, d, *J* = 8.9 Hz), 7.33-7.55 (3H, 7.39 (d, *J* = 8.7 Hz), 7.49 (d, *J* = 8.9 Hz)), 7.86 (1H, d, *J* = 8.7 Hz).

^{13}C NMR: δ 114.3 (2C, s), 124.0 (1C, s), 128.6 (2C, s), 131.0 (1C, s), 134.3 (1C, s), 134.7 (1C, s), 148.4 (1C, s), 151.1 (1C, s), 166.3 (1C, s).

IR(Cm^{-1})= 1600cm^{-1}(CO-stretching)

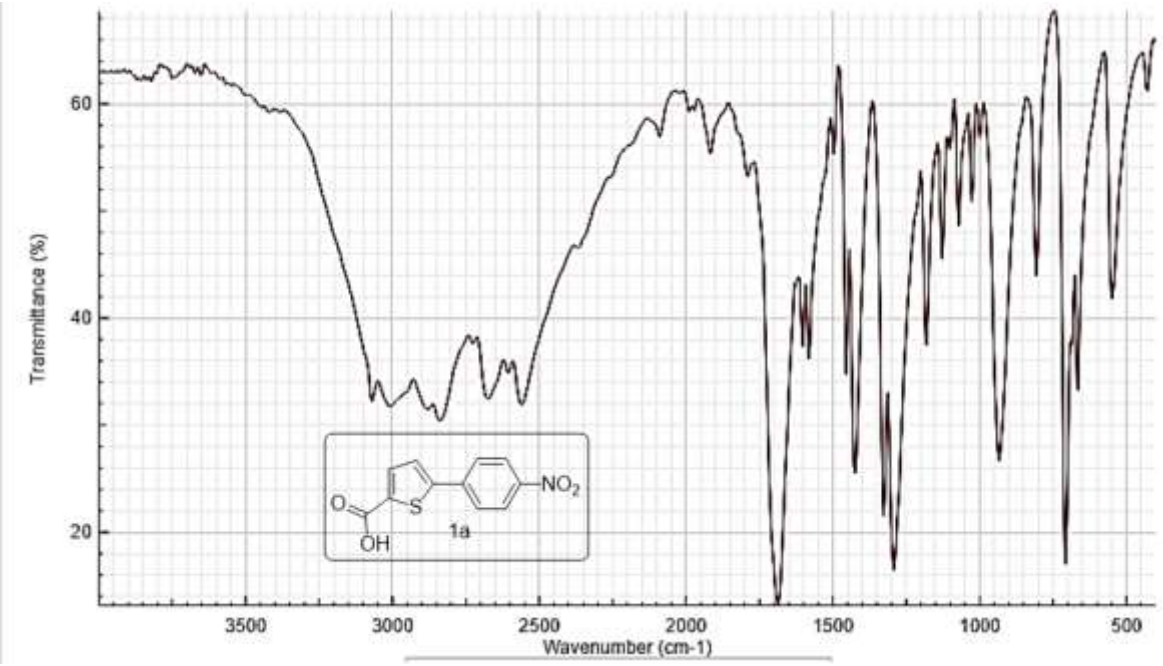


Fig:1 IR spectra of Compound 1a

Compound 2a

^{1}H NMR: δ 6.88 (2H, d, *J* = 9.0, 1.1, 0.4 Hz), 7.34-7.55 (3H, 7.40 (d, *J* = 8.7 Hz), 7.49 (d, *J* = 9.0, 1.5, 0.4 Hz)), 7.71 (1H, d, *J* = 8.7 Hz).

^{13}C NMR: δ 114.3 (2C, s), 124.0 (1C, s), 127.0 (1C, s), 128.6 (2C, s), 131.6 (1C, s), 134.3 (1C, s), 148.4 (1C, s), 151.1 (1C, s), 168.0 (1C, s).

Compound 3a

^{1}H NMR: δ 6.88 (2H, d, *J* = 9.0, 1.1, 0.4 Hz), 7.30-7.54 (3H, 7.37 (d, *J* = 8.7 Hz), 7.48 (d, *J* = 9.0, 1.5, 0.4 Hz)), 7.65 (1H, d, *J* = 8.7 Hz).

^{13}C NMR: δ 114.3 (2C, s), 124.0 (1C, s), 128.6 (2C, s), 129.6 (1C, s), 134.3-134.5 (2C, 134.3 (s), 134.4 (s)), 148.4 (1C, s), 151.1 (1C, s), 163.2 (1C, s), 181.7 (1C, s).

Compound 6a

^{1}H NMR: δ 2.20 (3H, s), 6.61 (1H, d, *J* = 9.0 Hz), 6.87 (2H, d, *J* = 8.2 Hz), 6.99-7.19 (3H, 7.05 (d, *J* = 8.2, Hz), 7.13 (d, *J* = 8.8Hz)), 7.22-7.49 (5H, 7.29 (d, *J* = 8.5 Hz), 7.31 (d, *J* = 8.5 Hz), 7.42 (d, *J* = 9.0, 1.5, 0.4 Hz), 7.42 (d, *J* = 8.8, 1.5, 0.4 Hz), 7.42 (d, *J* = 1.3 Hz)), 8.55 (1H, d, *J* = 1.3 Hz).

^{13}C NMR: δ 21.3 (1C, s), 114.3-114.3 (4C, 114.3 (s), 114.3 (s)), 121.8 (1C, s), 122.7 (1C, s), 124.0 (1C, s), 127.3 (1C, s), 128.1 (1C, s), 128.6 (2C, s), 129.6 (2C, s), 134.3 (1C, s), 136.0 (1C, s), 141.5 (1C, s), 147.3 (1C, s), 148.3-148.5 (2C, 148.4 (s), 148.4 (s)), 151.1 (1C, s)

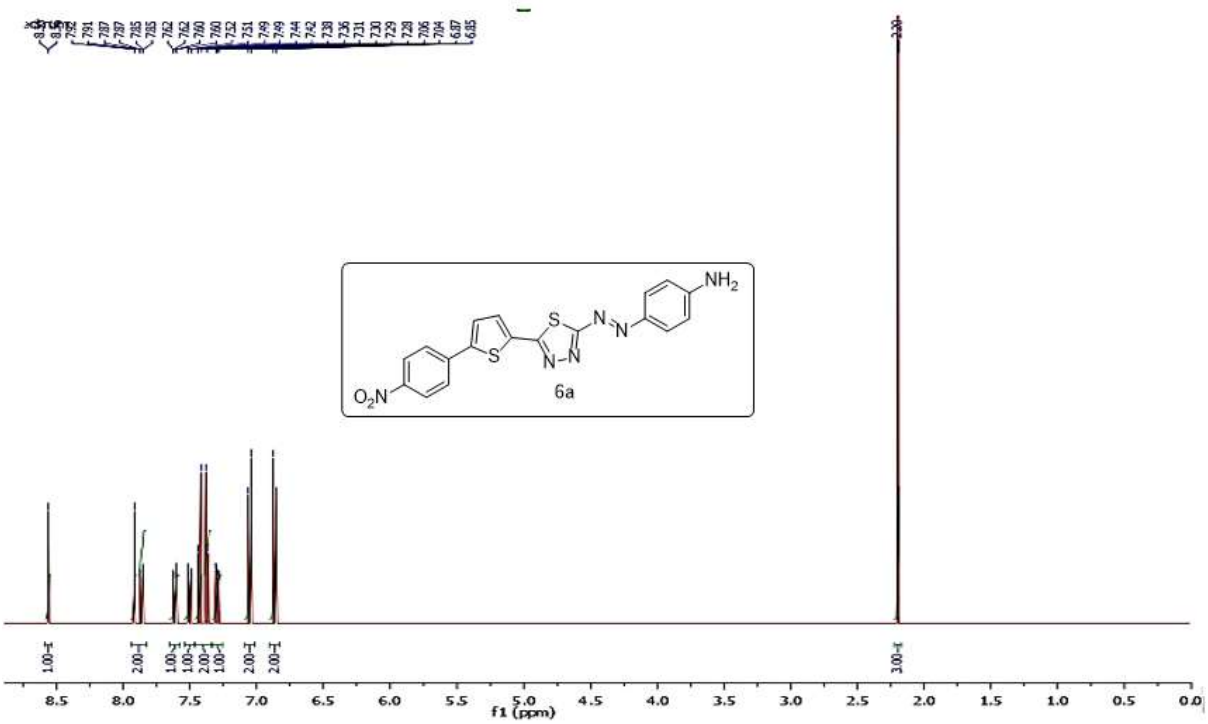


Fig:2 1HNMR spectra of Compound 6a

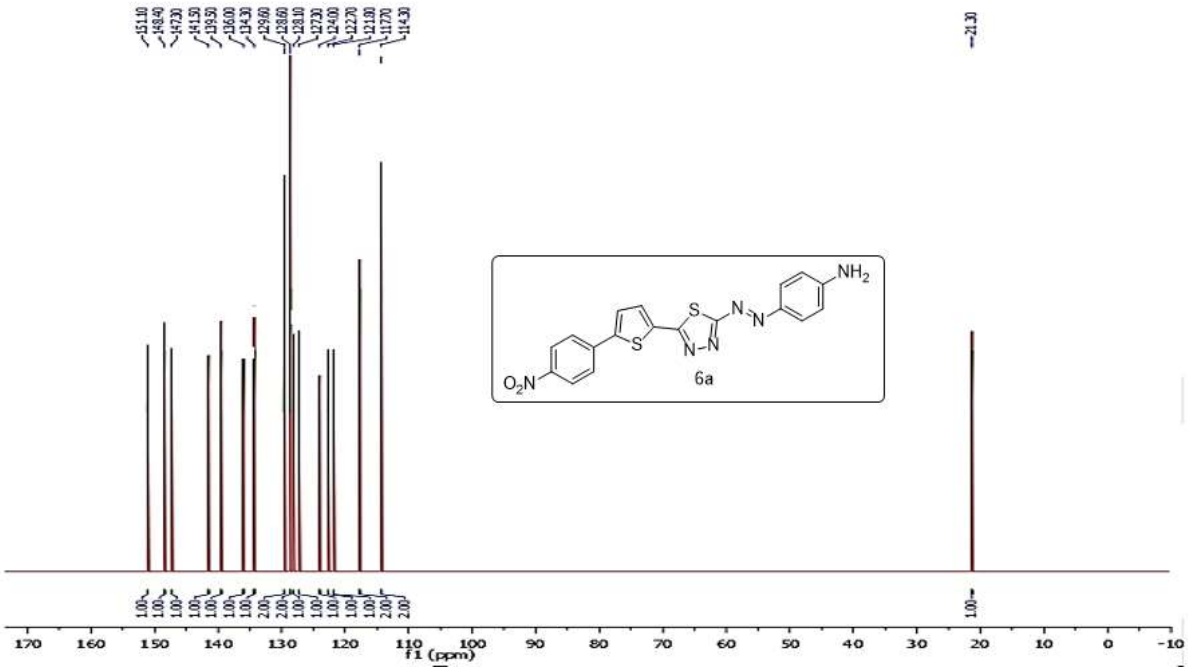


Fig:3 C13NMR spectra of Compound 6a

Compound 6b

^{1}H NMR: δ 2.20 (3H, s), 6.87 (2H, d, *J* = 8.2, 1.2, 0.5 Hz), 7.05 (2H, d, *J* = 8.2, 1.2, 0.5 Hz), 7.32 (1H, d, *J* = 8.5 Hz), 7.40-7.66 (5H, 7.46 (d, *J* = 8.9, 1.5, 0.5 Hz), 7.48 (d, *J* = 8.9, 1.5, 0.5 Hz), 7.50 (d, *J* = 8.8, 1.5, 0.5 Hz), 7.50 (d, *J* = 8.5 Hz), 7.59 (d, *J* = 8.8, 1.5, 0.5 Hz)), 7.98 (1H, d, *J* = 1.4 Hz), 8.57 (1H, d, *J* = 1.4 Hz).

^{13}C NMR: δ 21.3 (1C, s), 114.3 (2C, s), 121.8 (1C, s), 122.7 (1C, s), 124.0 (1C, s), 127.3 (1C, s), 127.6 (2C, s), 128.1 (1C, s), 128.7 (2C, s), 129.6 (2C, s), 133.7 (1C, s), 134.3 (1C, s), 136.0 (1C, s), 141.5 (1C, s), 147.3 (1C, s), 148.4 (1C, s), 151.1 (1C, s).

Compound 6c

^{1}H NMR: δ 2.20 (3H, s), 6.87 (2H, d, *J* = 8.2, Hz), 7.05 (2H, d, *J* = 8.2, Hz), 7.34-7.65 (6H, 7.40 (d, *J* = 8.9, Hz), 7.47 (d, *J* = 8.5 Hz), 7.50 (d, *J* = 8.5 Hz), 7.56 (d, *J* = 8.8, Hz), 7.58 (d, *J* = 8.8Hz), 7.58 (d, *J* = 8.9Hz)), 7.97 (1H, d, *J* = 1.4 Hz), 8.56 (1H, d, *J* = 1.4 Hz
^{13}C NMR: δ 21.3 (1C, s), 114.3 (2C, s), 121.8 (1C, s), 122.3 (1C, s), 122.7 (1C, s), 124.0 (1C, s), 127.3 (1C, s), 127.9 (2C, s), 128.1 (1C, s), 129.6 (2C, s), 131.7 (2C, s), 134.3 (1C, s), 136.0 (1C, s), 141.5 (1C, s), 147.3 (1C, s), 148.4 (1C, s), 151.1 (1C, s).

Compound 6d
^{1}H NMR: δ 2.20 (3H, s), 6.87 (2H, d, *J* = 8.2, 1.2, 0.5 Hz), 7.05 (2H, d, *J* = 8.2, 1.2, 0.5 Hz), 7.28-7.44 (2H, 7.34 (d, *J* = 8.5 Hz), 7.37 (d, *J* = 8.1, 7.8, 0.5 Hz)), 7.45-7.70 (3H, 7.51 (d, *J* = 8.5 Hz), 7.57 (d, *J* = 8.1, 1.5, 1.3 Hz), 7.63 (d, *J* = 7.8, 1.5, 1.3 Hz)), 7.75 (1H, td, *J* = 1.5, 0.5 Hz), 8.00 (1H, d, *J* = 1.4 Hz), 8.57 (1H, d, *J* = 1.4 Hz)
^{13}C NMR: δ 21.3 (1C, s), 114.3 (2C, s), 121.8 (1C, s), 122.7 (1C, s), 124.0 (1C, s), 127.0 (1C, s), 127.3 (1C, s), 127.7 (1C, s), 128.1 (1C, s), 128.7 (1C, s), 128.9 (1C, s), 129.6 (2C, s), 130.4 (1C, s), 133.0 (1C, s), 136.0 (1C, s), 141.5 (1C, s), 147.3 (1C, s), 148.4 (1C, s), 151.1 (1C, s).

Compound 6e
^{1}H NMR: δ 2.20 (3H, s), 6.87 (2H, d, *J* = 8.2, 1.2, 0.5 Hz), 6.99-7.38 (5H, 7.05 (d, *J* = 8.2, 1.2, 0.5 Hz), 7.13 (d, *J* = 8.9, 1.3, 0.5 Hz), 7.21 (d, *J* = 8.9, 1.3, 0.5 Hz), 7.32 (d, *J* = 8.5 Hz)), 7.49 (1H, d, *J* = 8.5 Hz), 7.59-7.73 (2H, 7.65 (d, *J* = 8.9, 1.5, 0.5 Hz), 7.67 (d, *J* = 8.9, 1.5, 0.5 Hz)), 7.96 (1H, d, *J* = 1.3 Hz), 8.56 (1H, d, *J* = 1.3 Hz).
^{13}C NMR: δ 21.3 (1C, s), 114.3 (2C, s), 115.4 (2C, s), 121.8 (1C, s), 122.7 (1C, s), 124.0 (1C, s), 127.3 (1C, s), 127.8 (2C, s), 128.1 (1C, s), 129.6 (2C, s), 134.3 (1C, s), 136.0 (1C, s), 141.5 (1C, s), 147.3 (1C, s), 148.4 (1C, s), 151.1 (1C, s), 162.5 (1C, s).

Compound 7a
^{1}H NMR: δ 2.17 (3H, s), 2.78 (6H, s), 6.61 (1H, d, *J* = 9.0Hz), 6.82 (2H, d, *J* = 8.2, Hz), 6.99-7.19 (3H, 7.05 (d, *J* = 8.2, Hz), 7.13 (d, *J* = 8.8Hz)), 7.22-7.49 (4H, 7.29 (d, *J* = 8.5 Hz), 7.31 (d, *J* = 8.5 Hz), 7.42 (d, *J* = 9.0, 1.5, 0.4 Hz), 7.42 (d, *J* = 8.8 Hz)), 7.99 (2H, d, *J* = 1.3 Hz)
^{13}C NMR: δ 21.3 (1C, s), 40.3 (2C, s), 112.0 (2C, s), 114.3 (2C, s), 121.8 (1C, s), 122.7 (1C, s), 124.0 (1C, s), 127.3 (1C, s), 128.1 (1C, s), 128.6 (2C, s), 129.6 (2C, s), 134.3 (1C, s), 136.0 (1C, s), 141.5 (1C, s), 147.3 (1C, s), 148.4 (1C, s), 150.9 (1C, s), 151.1 (1C, s)

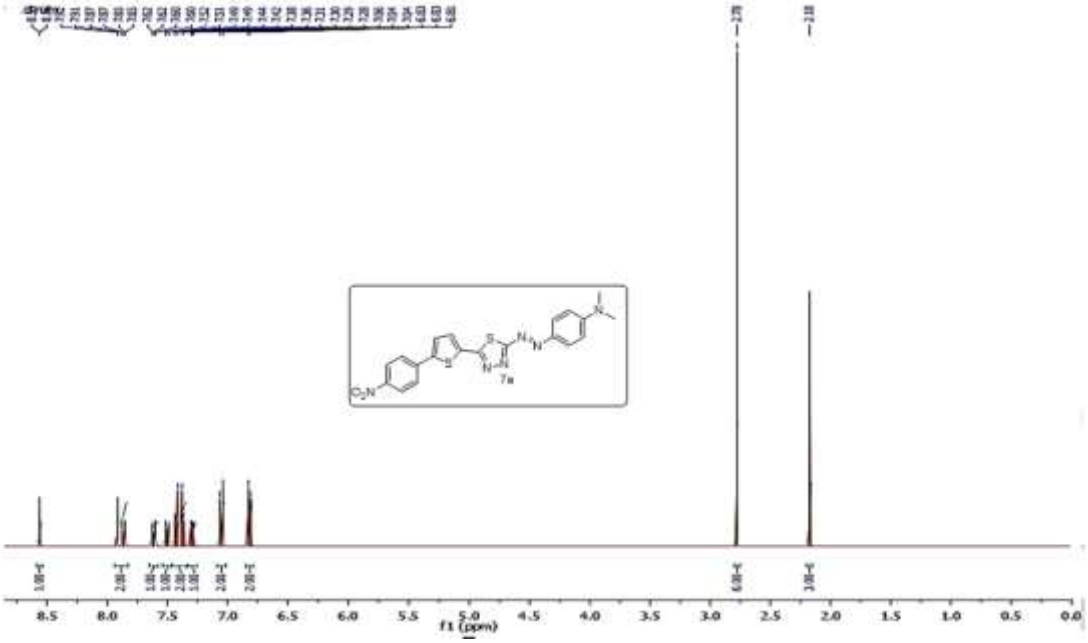


Fig:4 1HNMR spectra of Compound 7a

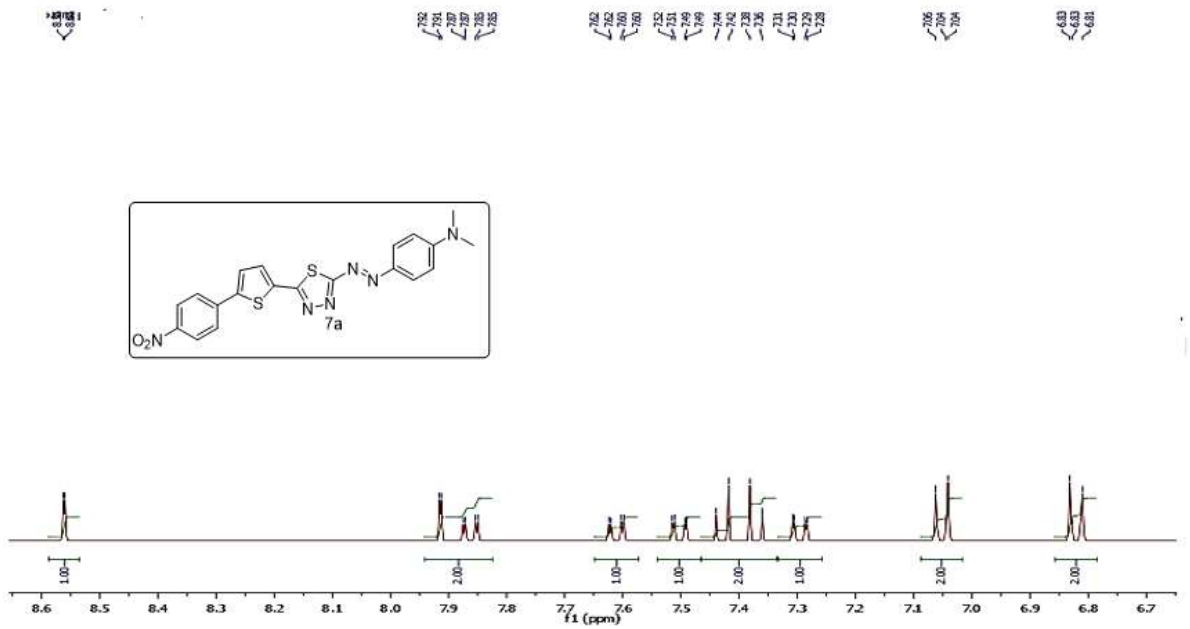


Fig:4a 1HNMR Expandable spectra of Compound 7a Aromatic region

Compound 7b

^{1}H NMR: δ 2.17 (3H, s), 2.78 (6H, s), 6.82 (2H, d, *J* = 8.2, 1.4, 0.5 Hz), 7.05 (2H, d, *J* = 8.2, Hz), 7.32 (1H, d, *J* = 8.5 Hz), 7.40-7.66 (5H, 7.46 (d, *J* = 8.9,Hz), 7.48 (d, *J* = 8.9, Hz), 7.50 (d, *J* = 8.8, Hz), 7.50 (d, *J* = 8.5 Hz), 7.59 (d, *J* = 8.8, Hz)), 7.98 (1H, d, *J* = 1.4 Hz), 8.57 (1H, d, *J* = 1.4 Hz).

^{13}C NMR: δ 21.3 (1C, s), 40.3 (2C, s), 112.0 (2C, s), 121.8 (1C, s), 122.7 (1C, s), 124.0 (1C, s), 127.3 (1C, s), 127.6 (2C, s), 128.1 (1C, s), 128.7 (2C, s), 129.6 (2C, s), 133.7 (1C, s), 134.3 (1C, s), 136.0 (1C, s), 141.5 (1C, s), 147.3 (1C, s), 150.9 (1C, s), 151.1 (1C, s)

Compound 7c

^{1}H NMR: δ 2.17 (3H, s), 2.78 (6H, s), 6.82 (2H, d, *J* = 8.2, 1.4, 0.5 Hz), 7.05 (2H, d, *J* = 8.2, Hz), 7.34-7.65 (6H, 7.40 (d, *J* = 8.9, Hz), 7.47 (d, *J* = 8.5 Hz), 7.50 (d, *J* = 8.5 Hz), 7.56 (d, *J* = 8.8, Hz), 7.58 (d, *J* = 8.8, Hz), 7.58 (d, *J* = 8.9, Hz)), 7.97 (1H, d, *J* = 1.4 Hz), 8.56 (1H, d, *J* = 1.4 Hz)

^{13}C NMR: δ 21.3 (1C, s), 40.3 (2C, s), 112.0 (2C, s), 121.8 (1C, s), 122.3 (1C, s), 122.7 (1C, s), 124.0 (1C, s), 127.3 (1C, s), 127.9 (2C, s), 128.1 (1C, s), 129.6 (2C, s), 131.7 (2C, s), 134.3 (1C, s), 136.0 (1C, s), 141.5 (1C, s), 147.3 (1C, s), 150.9 (1C, s), 151.1 (1C, s)

Compound 7d

^{1}H NMR: δ 2.17 (3H, s), 2.78 (6H, s), 6.82 (2H, d, *J* = 8.2, Hz), 7.05 (2H, d, *J* = 8.2, Hz), 7.28-7.44 (2H, 7.34 (d, *J* = 8.5 Hz), 7.37 (d, *J* = 8.1, 7.8, 0.5 Hz)), 7.45-7.70 (3H, 7.51 (d, *J* = 8.5 Hz), 7.57 (d, *J* = 8.1, Hz), 7.63 (d, *J* = 7.8, Hz)), 7.75 (1H, td, *J* = 1.5, Hz), 8.00 (1H, d, *J* = 1.4 Hz), 8.57 (1H, d, *J* = 1.4 Hz).

^{13}C NMR: δ 21.3 (1C, s), 40.3 (2C, s), 112.0 (2C, s), 121.8 (1C, s), 122.7 (1C, s), 124.0 (1C, s), 127.0 (1C, s), 127.3 (1C, s), 127.7 (1C, s), 128.1 (1C, s), 128.7 (1C, s), 128.9 (1C, s), 129.6 (2C, s), 130.4 (1C, s), 133.0 (1C, s), 136.0 (1C, s), 141.5 (1C, s), 147.3 (1C, s), 150.9 (1C, s), 151.1 (1C, s).

Compound 7e
^{1}H NMR: δ 2.17 (3H, s), 2.78 (6H, s), 6.82 (2H, d, *J* = 8.2, Hz), 6.99-7.38 (5H, 7.05 (d, *J* = 8.2, Hz), 7.13 (d, *J* = 8.9, Hz), 7.21 (d, *J* = 8.9, Hz), 7.32 (d, *J* = 8.5 Hz)), 7.49 (1H, d, *J* = 8.5 Hz), 7.59-7.73 (2H, 7.65 (d, *J* = 8.9, Hz), 7.67 (d, *J* = 8.9, Hz)), 7.96 (1H, d, *J* = 1.3 Hz), 8.56 (1H, d, *J* = 1.3 Hz).
^{13}C NMR: δ 21.3 (1C, s), 40.3 (2C, s), 112.0 (2C, s), 115.4 (2C, s), 121.8 (1C, s), 122.7 (1C, s), 124.0 (1C, s), 127.3 (1C, s), 127.8 (2C, s), 128.1 (1C, s), 129.6 (2C, s), 134.3 (1C, s), 136.0 (1C, s), 141.5 (1C, s), 147.3 (1C, s), 150.9 (1C, s), 151.1 (1C, s), 162.5 (1C, s).

Pharmacology
Azoles exert antifungal activity through inhibition based on the structure of the active site of thiadiazoles and extensive investigation of the structure–activity relationships (SAR) of azole has revealed that the thiazdiazole ring, having sulphur , nitrogen group was the pharmacophore of antimicrobial agents .

Evaluation of minimal inhibitory concentrations (MICs)
Table 1 In vitro minimum inhibition concentration evaluation of test compounds against Staphylococcus aureus, Pseudomonas aureginosa, Bacillus subtilis, Escherichia coli, Candida albicans and Candida parapsilosis

	6a	**6b**	**6c**	**6d**	**6e**
Staphylococcus aureus	20	10	05	10	20
Pseudomonas aureginosa	10	0.5	05	10	10
Bacillus subtilis	10	10	2.5	05	10
Escherichia coli	10	0.5	2.5	05	-
Candida parapsilosis	-	05	05	10	-

Antimicrobial screening
All synthesized compounds having heterocyclic system containing bridgehead nitrogen and oxygen atoms possess enhanced antimicrobial activity. Among the synthesized compounds 4c having moderate aliphatic chain length showed significant results in inhibiting Staphylococcus aureus and Bacillus subtilis growth with 21.83 ± 0.88 mm and 24.33 ±1.01 mm zones of inhibition respectively when compared to other compounds. Compounds 6b and 6a against P. aureginosa produced 21.01± 0.56 mm and 17.69 ± 0.33 mm zones of inhibition respectively; this was comparable to the effect of the standard used. Whereas compound 6d was significant and showed 17.5 ± 1.2 mm. Test compounds other than 6c showed moderate effect when compared to the standard drug ampicillin against E. coli. Compound 6c showed significant inhibition against Candida albicans and C. parapsilosis with 25.67 ±0.58 mm and 17.33 ± 1.01 mm zones of inhibition respectively when compared to other compounds but less efficient than the standard drug fluconazole. Evaluation of antimicrobial activity revealed that the all the synthesized compounds were effective in inhibiting the bacterial and fungal growth but with some exceptions. Among all the compounds tested, compounds 6b, 6d, and 6a showed significant antimicrobial activity when compared to other compounds. Specifically compound 6c was more efficient than other compounds but less potent than standard drug ampicillin. Results of in vitro antimicrobial activity are depicted in Table 2.

Determination of minimal inhibitory concentrations (MIC)

The agar dilution susceptibility test was performed based on the modified method of NCCLS, 2003 and CLSI, 2009 to determine the MIC of the synthesized compounds. The test compounds 6(a-e) dissolved in sterilized 5% DMSO (400 mg/mL concentration) were taken as standard stock. A series of two fold dilutions of each compound in the final concentrations of 40, 20, 10, 5, and 2.5 mg/mL were prepared in nutrient agar for bacteria and potato dextrose agar for fungi. After solidification, the plates were spotted with 100 lL of overnight grown bacterial cultures approximately containing 1 104 CFU/mL. The test was carried out in triplicates. The plates of bacterial culture were incubated at 37^0 C for 18– 24 h and fungal cultures were incubated at 24^0 C for 24– 48 h. After incubation, the MIC was determined.

Table 2 Antimicrobial activity of the synthesized compounds 6(a-e) against Staphylococcus aureus, Pseudomonas aureginosa, Bacillus subtilis, Escherichia coli and Candida parapsilosis

Compounds	Zone of inhibition (mm)				
	Staphylococcus aureus	**Bacillus subtilis**	**Escherichia coli**	**Pseudomonas aeruginosa**	**Candida parapsilosis**
6a	10.33±1.12	16.67± 0.95	17.88±1.53	17.69±0.33	ND
6b	17 ±1.53	17.67± 0.88	18.83±1.4	21.01±0.56	18.01±0.33
6c	21.83±0.88	24.33± 1.01	23±0.33	20±0.33	17.33±1.01
6d	17.33±0.67	18.83± 0.33	18.33±0.88	17.5±1.2	16.98±1.13
6e	12.28±0.88	11.67± 0.33	17.17±1.45	15.11±0.33	15.17±1.52
Ampicillin	17 ±1.53	20.67± 0.33	14.33±1.45	19.67±0.88	-
Fluconazole	-	-	-	-	18.83±1.13

The tested compounds were dissolved in dimethyl sulphoxide (DMSO) at concentration of 100 and 50 mg/ml. Approximately 1 cm^3 of a 24 h broth culture was placed in sterile petri dishes. Molten nutrient agar kept at 45^0 C was then poured into the Petri dishes and allowed to solidify. Six millimetre diameter holes were then punched carefully using a sterile cork borer and completely filled with the test solutions. The plates were incubated for 24 h at 37^0 C. The inhibition zone was observed and documented after 24 h for bacterial culture and 72 h for fungal cultures. The clear zone around the holes in each plate was measured as zone of inhibition in mm. Experiments were triplicates and standard deviation was calculated.

Result and Discussion

As depicted in Scheme 1, 1,3,4-thiadiazole azo dye derivatives were synthesized by a multi-step reaction sequence. 1,3,4- thiadiazole were prepared by taking thiophene Aldehyde as a starting material it was prepared by diazotisation process finally it is converted to carboxylic acid ,it is made to react with thionyl chloride to obtain 2-(5-(Substitutedphenyl)thiophene-2-carbonyl)hydrazine-1-carbothioamide.again it is made to react with sulphuric acid to obtain 5'-(Substituted phenyl)-[2,2'-bithiophen]-5-amine. Finally after diazotisation process it was made to react with anilines and dimethyl anilines to obtaindimethyl-4-((5'-(Substitutedphenyl)-[2,2'-bithiophen] 5yl)diazenyl)aniline. The synthesized compounds were recrystallized using methanol. The purity of the compounds was checked by TLC. The structures of the newly synthesized compounds were characterized by 1H NMR, IR and Mass spectra studies. The

synthesized compounds were found in good agreement with the spectral data. Confirmation of Carboxylic acid was done by IR spectra we obtained 1600cm^{-1} as stretching frequency due to presence of carbonyl group .Finally thidiazoles were confirmed by 1HNMR spectra observed chemical shift around 2.17ppm it confirms the methyl group and we observed chemical shift around 7.00-8.00ppm due to presence of aromatic group and finally NH peak were observed at 8.5ppm. The elemental analysis results were matched within ±0.4% of the theoretical values. The IR, 1H NMR and mass spectral data were found in good agreement with the newly synthesized compounds.

Conclusion

This investigation proposes a convenient, economical, cheaper and useful method for the synthesis of 1,3,4-Thiadiazole-Containing azo Dyes which are biologically active molecules possessing antimicrobial activities. These new classes of heterocycles, exhibit a significant antimicrobial activities. The preliminary antimicrobial activity studies revealed that the azo dye having 1,3,4-thiazdiazole moiety exhibited a potential antimicrobial activity. Hence, it can be concluded that, this new class of compounds certainly holds a greater promise in discovering a potent antimicrobial agent.

Conflicts of Interest

The authors declare no conflicts of interest.

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