Research Article

Synthesis and Biological Evaluation of Novel 1,3,4 Thia di azole Heterocyclic Derivatives as Novel Anti-Microbial Agents

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ABSTRACT

Heterocyclic compounds are commonly used Scaffolds on which pharmacophores are arranged to provide potent and selective drugs. This is especially true for five-membered ring heterocyclic compounds, which serve as the core components of many substances that possess a wide range of interesting biological activities. New series of 1,3,4 Thia di azole derivatives were synthesized From Benzyl Isocynate and Acid hydrazide, by using P-TsCl in NMP, The Novel Derivatives have been screened for their antimicrobial activity.

Keywords: 1,3,4 Thia di azoles, Synthesis, Anti-bacterial activity and Anti- Fungal activity.

INTRODUCTION

Heterocyclic Compounds have so far been Synthesized Mainly due to the wide range of Biological Activities[1]. Over the past decades, the bulk of chemists' interests have been on Heterocyclic compounds and their various derivatives as well as their applications in the pharmaceutical and chemical fields.

Research concerning many kinds of Heterocyclic compounds, such as pyrazole, tetrahydroquinolines, benzotriazole, 1,2,3,4-tetrazine, thiazole, 2-thiazoline, pyrimidine, and so on, has been the subject of numerous recent reviews.

Thiadiazole is a prevalent and important five-membered Heterocyclic system containing two nitrogen atoms and a sulfur atom.

There are several isomers of thiadiazole including 1,2,3thiadiazole, 1,2,4-thiadiazole, 1,2,5-thiadiazole, and 1,3,4thiadiazole (**Figure 1**).

A glance at standard reference works shows 1,3,4-thiadiazole has been investigated more than other isomers. The 1,3,4thiadiazole ring is a very weak base due to the inductive effect of the sulfur atom and possesses relatively high aromaticity. It is 1,3,4-Thiadiazole derivatives possessed a wide range of therapeutic activities like antimicrobial[2], antifungal[3], anti-micro bacterial[4], antileshmanial[5], analgesic, antiinflammatory[6], antidepressant[7], antipsychotic[8] and anticonvulsant[8,9]. 1,3,4-Thiadiazole derivatives exhibited interesting *in vitro*[10-12] and *in vivo*[13-16] antitumor activities. Different mechanisms of action were attributed to antitumor activity of 1,3,4-thiadiazole ring such as inhibited DNA and RNA syntheses specifically without appreciably affecting protein synthesis[17], inhibition of carbonic anhydrase[18], phosphodiesterase-7 (PDE7)[19], histone deacetylase[20] or as adenosine A3 receptor antagonists[21].

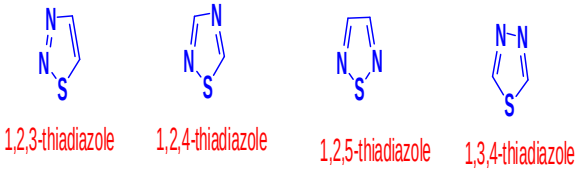


Figure 1: Isomers of thiadiazole.

Encouraged by the diverse biological activities of 1,3,4 Thia di azole Heterocyclic compounds, it was decided to prepare a new series of 1,3,4 Thia di azole derivatives. In the present communication, 1,3,4 Thia di azole derivatives 4 (a-f) were prepared by the action of substituted isocynates (1) with acid hydrazides (2) in the presence of aqueous solution of TEA at room temperature in presence of TEA to obtained Intermediate derivative (3). The Intermediate derivative (3) was reacted with TEA, P-TsCl, in NMP to afford Novel 1,3,4 Thia di azole derivatives4(a-f). Scheme 1. The structures of all synthesized compounds were assigned on the basis of IR, Mass, ^{1}H NMR spectral data. Further these compounds were subjected for antifungal and antibacterial activity.

MATERIALS AND METHODS

Laboratory chemicals were provided by Rankem India Ltd. and Ficher Scientific Ltd. Melting points were determined by the open tube capillary method and are not correct. The purity of the compounds was determined by thin layer chromatography (TLC) plates (silica gel G) in the solvent system toluene:ethyl acetate (8:2). The spots were observed by exposure to iodine Vapours or by UV light or P-Anisaldehyde Stain Solution. The IR spectra were received by PerkineElmer 1720 FT-IR spectrometer (KBr pellets). The ^{1}H NMR & ^{13}C NMR spectra were obtained by Bruker Advance II 400 spectrometer using TMS because the internal standard in $CDCl_3$. Elemental analysis of the new synthesized compounds were

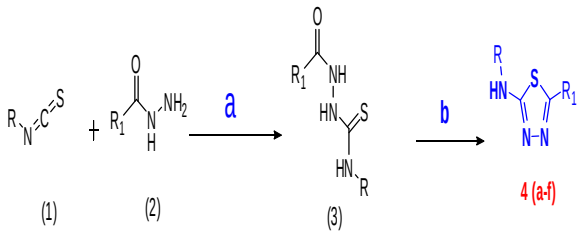
obtained by Carlo Erba 1108 analyzer. General Information. Commercial chemicals were treated as follows: DMF, distilled from CaH_2 and degassed (freeze and thaw) three times prior to use; THF, ether, hexanes distilled from Na/benzophenone.

The synthesis of the compounds as per the following **Scheme I** given below.

The synthetic route was depicted in scheme **I**

The title compounds 4(a-f) were synthesised in two sequential steps using different reagents and reaction conditions, the 4(a-f) were obtained in moderate yields. The structure were established by spectral (IR, ^{1}H-NMR, ^{13}C-NMR and mass).

Synthetic Scheme



Reagents & Reaction Conditions : (a) TEA, Dry THF, RT (b) TEA, P-TsCl, NMP, 0^0C-RT, 4 hrs

S. No.	Compound Code	R	R_1
1	4a	-Bn	-Ph
2	4b	-Bn	-4F -Ph
3	4c	-Bn	-4 NO_2 -Ph
4	4d	-4 OMe -Bn	-Ph
5	4e	-4 CF_3 -Bn	-Ph
6	4f	-4 F -Ph	-Ph

Experimental Section

All reactions were carried out under argon in oven-dried glassware with magnetic stirring. Unless otherwise noted, all materials were obtained from commercial suppliers and were used without further purification. All solvents were reagent grade. THF was distilled from sodium benzophenone ketyl and degassed thoroughly with dry argon directly before use. Unless otherwise noted, organic extracts were dried with anhydrous Na_2SO_4, filtered through a fitted glass funnel, and concentrated with a rotary evaporator (20–30 Torr). Flash chromatography was performed with silica gel (200–300 mesh) by using the mobile phase indicated. The NMR spectra were measured with a 400 MHz Bruker Avance spectrometer at 400.1 and 100.6 MHz for ^{1}H for ^{13}C, respectively, in $CDCl_3$ solution with tetra methyl silane as internal standard. Chemical shifts are given in ppm (δ) and are referenced to the residual proton resonances of the solvents. Proton and carbon magnetic resonance spectra (1H NMR and ^{13}C NMR) were recorded using tetramethylsilane (TMS) in the solvent of $CDCl_3$-d or DMSO-d_6 as the internal standard (^{1}H NMR: TMS at 0.00 ppm, $CDCl_3$ at 7.26 ppm, DMSO at 2.50 ppm; ^{13}C NMR: $CDCl_3$ at 77.16 ppm, DMSO at 40.00 ppm).

General procedure for the preparation of Thiosemicarbazide (3a–3f)

Benzyl-isothiocyanate 1 (**2.40 m.mol**) was added to a stirred solution of benzoylhydrazide 2 (**2.00 m.mol**) and triethylamine (**2.00 m.mol**) in 10 mL of THF. The reaction mixture was stirred at room temperature for 16 h, and then the solvents were removed via a rotary evaporator. The residue was triturated with diethyl ether/ethyl acetate (95:5) to afford 95% yield, of the desired thiosemicarbazide.

General procedure for the preparation of 2-amino1,3,4-thiadiazole (4a-4f)

p-TsCl (**0.60 m.mol**) was added to a stirred solution of thiosemicarbazide 3a-3i (**0.50 m.mol**) and triethylamine (**1.10 m.mol**) in 4 mL of NMP. The reaction mixture was stirred at room temperature for 2 h and extracted with DCM (15 mL) and distilled water (10 mL), after which the aqueous layer was removed. The aqueous layer was back-extracted with DCM (3 × 10 mL). The combined organic layers were dried over Na_2SO4 and evaporated to afford the crude product, which was purified by column chromatography on silica gel (hexane/ethyl acetate) to afford 50-55 % yield of a 2-amino-1,3,4thiadiazole derivatives.

Spectral and Physical data of Novel 1,3,4 Thia diazole heterocyclic derivatives

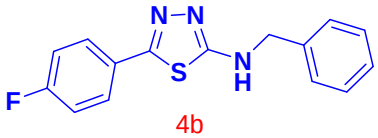
Compound 4a

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82–7.76 (m, 2H), 7.43–7.31 (m, 8H), 5.72 (s, 1H), 4.60 (s, 2H).

^{13}C NMR (101 MHz, CDCl3) δ 169.5, 158.4, 137.0, 131.0, 123.0, 128.9, 128.9, 128.1, 127.8, 126.9, 50.8.

Mp 180–181 °C.

N-Benzyl-5-(4-fluorophenyl)-1,3,4-thiadiazol-2-amine (4b)



4b

Yield, 82% (white solid).

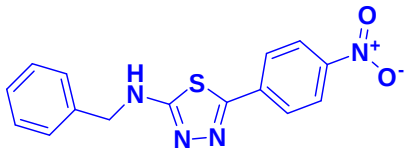
^{1}H NMR (400 MHz, DMSO) δ 8.45 (t, J = 5.8 Hz, 1H), 7.87–7.75 (m, 2H), 7.45–7.24 (m, 7H), 4.55 (d, J = 5.7 Hz, 2H).

^{13}C NMR (101 MHz, DMSO) δ 169.0, 163.2 (1JCF = 248.5 Hz), 155.6, 139.0, 119.9 (3JCF = 9.1 Hz), 128.9, 128.0, 127.9 (4JCF = 3.0 Hz), 127.6, 116.7 (2JCF = 22.2 Hz); 48.5.

Mp 173– 175 °C.

MS (ESI): m/z = 284.0 [M−1].

N-Benzyl-5-(4-nitrophenyl)-1,3,4-thiadiazol-2-amine (4c).



4c

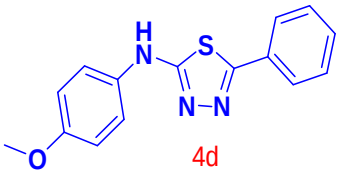
Yield, 60% (yellow solid).

^{1}H NMR (400 MHz, DMSO) δ 8.74 (t, J = 5.7 Hz, 1H), 8.30 (d, J = 8.9 Hz, 2H), 8.02 (d, J = 8.9 Hz, 2H), 7.46–7.24 (m, 5H), 4.59 (d, J = 5.8 Hz, 2H).

^{13}C NMR (101 MHz, DMSO) δ 170.2, 154.5, 148.0, 138.7, 137.1, 128.9, 128.1, 127.8, 127.6, 124.9, 48.6.

Mp 198–200 °C. MS (ESI): m/z = 310.9 [M−1].

N-(4-Methoxyphenyl)-5-phenyl-1,3,4-thiadiazol-2-amine (4d).



4d

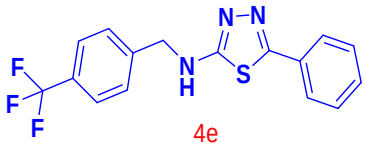
Yield, 53% (white solid).

^{1}H NMR (400 MHz, DMSO) δ 7.83 (dd, J = 6.9, 1.9 Hz, 2H), 7.47–7.40 (m, 3H), 7.39–7.32 (m, 2H), 6.95 (d, J = 8.7 Hz, 2H), 3.83 (t, J = 2.1 Hz, 3H).

^{13}C NMR (101 MHz, DMSO) δ 169.3, 159.9, 151.1, 130.7, 130.3, 129.0, 128.6, 127.6, 126.4, 114.9, 55.8.

Mp 227–229 °C. MS (ESI): m/z = 282.0 [M−1].

5-Phenyl-N-(4-(trifluoromethyl)benzyl)-1,3,4-thiadiazol-2amine (4e).



4e

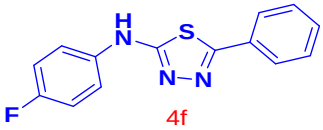
Yield, 65% (white solid).

^{1}H NMR (400 MHz, DMSO) δ 8.57 (t, J = 5.9 Hz, 1H), 7.81–7.70 (m, 4H), 7.61 (d, J = 8.1 Hz, 2H), 7.51–7.41 (m, 3H), 4.66 (d, J = 5.6 Hz, 2H).

^{13}C NMR (101 MHz, DMSO) δ 168.7, 157.1, 144.1, 131.2, 130.2, 129.6, 128.6, 126.9, 128.2 (2JCF = 31.3 Hz), 125.7 (3JCF = 3.8 Hz), 124.8 (1JCF = 273.7 Hz), 47.9.

Mp 153–155 °C. MS (ESI): m/z = 333.9 [M−1].

N-(4-Fluorophenyl)-5-phenyl-1,3,4-thiadiazol-2-amine (4f).



4f

Yield, 44% (white solid).

^{1}H NMR (400 MHz, DMSO) δ 9.34 (s, 1H), 7.88–7.82 (m, 2H), 7.48–7.41 (m, 5H), 7.15–7.08 (m, 2H).

^{13}C NMR (101 MHz, DMSO) δ 169.1, 162.5 (d, 1JCF = 248.0 Hz), 151.1, 131.6 (d, 3JCF = 9.4 Hz), 131.3 (d, 4JCF = 3.1 Hz), 130.8, 129.0, 128.8, 126.2, 116.7 (d, 2JCF = 22.6 Hz).

Mp 247–250 °C. MS (ESI): m/z = 269.9 [M−1].

Anti-Microbial Activity

Media and chemicals

Nutrient Broth, Nutrient agar and 5 mm diameter antibiotic assay were obtained from Hi-Media Laboratories Limited, India. Barium chloride dehydrate GR, concentrated sulphuric acid GR, Dimethyl sulphoxide GR, Sodium chloride AR and Potassiumdichromate were obtained from Ranbaxy Laboratories Ltd, Chemical Division, India. The standard bacterial and fungal strains were procured from National Centre from Cell Science (NCCS), Pune, India. The bacterial included two Gram positive bacterial isolates Staphylococcus aureus NCCS 2079 and Bacillus cereus NCCS 2106 and two Gram negative bacterial isolates Escherichia coli NCCS2065 and Pseudomonas aeruginosa NCCS 2200. The fungicidal organisms included were Aspergillus nigeri NCCS 1196 (AN) and Candida albicans NCCS 3471(CA). The bacteria were grown and maintained on nutrient agar (Hi-Media, Mumbai) and were subculture when needed.

Glass wares and Apparatus

Glass petridish, Glass tubes, Beakers, Erlenmeyer flasks, Bacterial loop and measuring cylinder. All the glass wares were of Borosilicate grade. Digital electronics balance (Shankar Scientific supplies, India), Yorco Horizontal Laminar air flow bench (Yorco sales Pvt. Ltd, New Delhi, India), Ausco incubator, Zone reader (Cintex industrial Corporation, India), hot air oven, autoclave and UV/Visible spectrophotometer (Shimadzu corporation, Japan).

Antibacterial activity

The antibacterial activity of synthesized compounds was studied by the disc diffusion method against the following pathogenic organisms. The gram-positive bacterial screened were Staphylococcus aureus NCCS 2079 (SA) and Bacillus cereus NCCS 2106 (BC). The gram negative bacterial screened were Escherichia coli NCCS 2065 (EC) and Pseudomonas aeruginosa NCCS 2200 (PA). The synthesized compounds were used at the concentration of 250 µg/ml and 500 µg/ml using DMSO as a solvent. The amoxicillin 10 µg/disc and Streptomycin 30 µg/disc were used as a standard (Himedia laboratories limited, Mumbai).

Disc Diffusion Method

A suspension of Staphylococcus aureus (SA) was added to sterile nutrient agar at 45°C. The mixture was transferred

to sterile petridishes to give a depth of 3 to 4 mm and allowed to solidify. Precautions were observed to reduce uniform layer of medium on the plate. Sterile discs 5mm in diameter (made from Whatman Filter paper) were immersed in the solutions of synthesized compounds (250µg/ml) and maintain an untreated control sample for comparison. Leave the plates to stand for 1hour at room temperature as a period of preincubation diffusion to minimize the effects of variations in different time. Then the plates were incubated at 37°C for 24 hours and observed for antibacterial activity. The diameter of the zone of inhibition was measured for each plate in which the zone of inhibition was observed. The average zone of inhibition was calculated and compared with that of standard. A similar procedure was adopted for studying the antibacterial activity against the other organisms.

Antifungal activity

The antifungal activity3 of synthesized compounds were studied by disc diffusion method against the organisms of Aspergillus nigeri NCCS 1196 (AN) and Candida albicans NCCS 3471(CA). Compounds were treated at the concentrations of 250 µg/ml using DMSO as a solvent. The standard used was Ketaconazole 50 µg/ml and Griseofulvin 50 µg/ml against both the organisms.

Disc Diffusion Method

A suspension of Aspergills nigeri NCCS 1196 (AN) was added to a sterile sabouraud dextrose agar at 45°C. The mixture was transferred to sterile petridishes and allowed to solidify. Sterile discs 5 mm in diameter (made from Whatmann Filter paper) immersed in the solutions of synthesized compounds and control were placed on the surface of agar medium with forceps and pressed gently to ensure even contact. Leave the plates to stand for 1 hour at room temperature as a period of preincubation diffusion to minimize the effects of variation at 37°C for 13 hours and observed for antibacterial activity. The diameters of the zone of inhibition were measured for the plates in which the zone of inhibition was observed. The average zone of inhibition was calculated with that of standard. The Novel 1,3,4 Thiadiazole derivates containing $–CF_3$ (4e) and -F atom (4f) showed more activity than other substituent's.

Antimicrobial evaluation of Novel compounds 4 (a-f):

S. No	Compound Code	R	R_1
1	4a	-Bn	-Ph
2	4b	-Bn	-4F -Ph
3	4c	-Bn	-4 NO_2 -Ph
4	4d	-4 OMe -Bn	-Ph
5	4e	-4 CF_3 -Bn	-Ph
6	4f	-4 F -Ph	-Ph

The order of activity was **4e>4f>4b>4c>4a>4d**.

Table 2: Antimicrobial activity and antifungal activity of synthesized compounds 4(a-f)

Compound No	Zone of inhibition in mm					
	Antibacterial activity			Antifungal activity		
	S.aureus	E.coli	P.aeruginosa	C. albicans	A. flavus	A.fumigatus
4a	20	17	18	10	9	10
4b	22	20	21	10	9	10
4c	21	18	19	10	9	10
4d	19	17	17	11	10	11
4e	24	22	23	12	10	11
4f	23	21	22	11	9	10
Ampicillin	20	21	22	21	-	-
Flucanazole	22	20	23	22	-	--

RESULTS AND DISCUSSION

The title compounds 4a-4f were synthesized in good yields (scheme-I). All these compounds were tested for anti-bacterial and anti-fungal activity showed considerable activity when compared to the standard drug Amoxicillin. It is interesting to note that the compound **4e, 4f** possessed the maximum activity. It clearly indicates the favourable effect of electron with drawing substituent's on the anti-bacterial and anti-fungal activity of the Novel 1,3,4 Thiadiazole Heterocyclic derivatives.

Readily available starting materials and simple synthesizing procedures make this method very attractive and convenient for the synthesis of Novel 1,3,4

Thiadiazole Heterocyclic derivatives. Formation of products was confirmed by recording their ^{1}H NMR, ^{13}C, FT-IR.

Biological Activity screening

The results of biological studies of newly synthesized compounds reveal that the compounds possess significant anti-bacterial and anti-fungal activities. The results of these studies are given in **Table 8**. From Anti-bacterial and Anti-fungal activity screening results, it has been observed that compounds **4e, 4f** possess good activity.

CONCLUSION

The approach of the present study was to synthesize various Novel 1,3,4 Thiadiazole derivatives and evaluate the anti-bacterial and anti-fungal activities. From result generated it can be concluded that test compounds 4a, 4b, 4c, 4d were found to possess moderate antibacterial activity against gram positive bacteria and gram negative bacteria compared with Amoxicillin. The observed antimicrobial and antifungal activities are attributed to the substitution of $–CF_3$ group & Fluorine atom in Novel 1,3,4 Thiadiazole compounds. The data reported in this article may be helpful guide for the medicinal chemist as well as Synthetic Chemist who is working in this area.

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