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Synthesis and antibacterial activity study of some new 1,3,4-thiadiazole derivatives

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ABSTRACT

Six substituted N-aryl,5-substituted phenyl 1,3,4-thiadiazole(7a-f) were synthesized by the reaction of different substituted benzaldehyde with different substituted 5-phenyl 1,3,4-thiadiazole 2-amino in the presence of sulphuric acid in refluxing methanol. The newly synthesized compounds were characterized by spectroscopic methods. Further, the synthesized compounds were screened for antibacterial and antifungal activity by standard method. Results of the activities reveal that some compounds exhibited moderate to good antimicrobial activity.

Keywords: Antimicrobial, Antifungal, 1,3,4-Thiadiazole derivatives.

INTRODUCTION

Medicinal chemistry is the application of chemical research techniques to the synthesis of pharmaceuticals. Heterocyclic chemistry is a vast and expending area of chemistry because of the obvious application of compound derived from heterocyclic rings in pharmacy medicine, agriculture,photography, rocket propellant as veterinary products as poetical bighting agent as antioxidant as corrosion inhibitors and also the other field. The synthesized heterocyclic compounds are screened for their possible antimicrobial and pharmacological activities. About half of the known compounds have structure that incorporates at least one heterocyclic compound [1]. Thiadiazole is a 5-membered ring system having two nitrogen and one sulphur atom. It is a versatile moiety having wide variety of pharmacological activities. It act as a constrained pharmacophore and as a hydrogen binding domain and two electron donating system. Thiadiazole and substituted thiadiazole are very much popular to medicinal chemist due to their wide range of diversified biological activity e.g. antimicrobial[2], anti-inflammatory[3], anticancer[4], anticonvulsant[5], antidepressant[6], antioxidant[7], radio protective[8], and anti-leishmanial[9], antiviral[10], diuretic[11],wound healing[12], and anti fungal activities[13].

MATERIALS AND MEHTODS

Melting point of the synthesized compounds were determined using melting point apparatus i.e. Temp Star, Hindustan Scientific Linkers and uncorrected. The solvents and reagents were used as received or dried prior to use as needed. All reactions were monitored and purity of compound checked by TLC using silica gel G as a stationary phase. The spots resolved were visualized as brown colored spots by using UV 256 detector. The IR spectra of the synthesized compounds were recorded using KBr pellets in range of 4000-500 cm^{-1}on IR spectrometer, Simazdu. ^{1}H-NMR(300 Mhz) spectra was recorded in DMSO-d_6 in BRUKER DPX-300 NMR spectrophotometer using Tetramethylsilane as internal standard.The mass spectra was recorded Perkin-Elmer Hitachi RMU-6L MS-30 spectrometer at 70 ev and a 90 ^{0}C inlet temperature.

Synthesis of Substituted Ethyl benzoate: Ethyl benzoate was prepared by refluxing benzoic acid with absolute alcohol in presence of sulphuric acid for 10-12 hrs. The mixture was cooled and then the solid mass was separated

by filtration and dried. It wasrecrystalized from chloroform- ethanol.For compound **3a** Melting point 155°C; yield-65%, R_f – 0.76 and for compound **3b** Melting point 156°C, yield-69%, R_f – 0.75.

Synthesis of Substituted Benzohydrazide: Ethyl benzoate was refluxed with Hydrazine hydride in presence of methanol for 8-10 hrs. The mixture was cooled and solid mass was collected by filtration and dried. It wasrecrystalized from chloroform- methanol.For compound **4a** Melting point 167°C, yield-72%, R_f – 0.87 and for compound**4b** Melting point 178°C, yield-68%, R_f – 0.64.

Synthesis of Substituted N-CarbamothioylBenzamide: Benzohydrazide was refluxed with Potassium thiocyanide in presence of methanol for 5-6 hrs. The mixture was cooled and the solid mass was collected by filtration and dried. It was recrystailzed from chloroform-methanol.For compound **5a** Melting point 175°C; yield-55%, R_f – 0.47 and for compound**5b** Melting point 189°C, yield-73%, R_f – 0.46.

Synthesis of Substituted 5-Phenyl-1,3,4- thiadiazole-2-amine: N-carbamothioylbenzamide and 5 ml of concentrated sulfuric acid was kept in room temperature for 5 -6 hrs in a closed glass container. Whole mass was poured into ice-water and the solid mass was collected by filtration and dried. Then obtained mass was recrystalized from rectified spirit-chloroform. For compound **6a**Melting point 183°C; yield-59%, R_f – 0.56 and for compound **6b** Melting point 200°C, yield-70%, R_f – 0.5. **6a** IR (KBr) cm^{-1} : 3216 (NH), 2914(C-H), 1656 (C=N), 1567(C=C), 845(N-N), 688(C-S); 1HNMR (DMSO) δppm: 5.24(s,2H, NH), 7.16-7.95(m, 9H, Ar-H), 8.34(s, 1H, N=CH-); MS (m/z) : 177(M+).

Synthesis of Substituted(Z)-2-(((5-phenyl-1,3,4-thiadiazol-2-yl)imino)methyl)phenol:
5-Phenyl-1,3,4- thiadiazole-2-amine was refluxed with salicyldehyde/p-Nitrobenzaldehyde/p-Chlorobenzaldehyde in presence of methanol for 10-12 hrs.The mixture was cooled and the solid mass was collected by filtration and dried to obtain the compounds 7a-f. It was recrystailzed from chloroform-methanol.

For compound **7a** Melting point 150-155°C, yield-68%, R_f – 0.51,IR (KBr)cm^{-1} : 3600(OH), 2916C-H), 1657 (C=N), 1568(C=C), 847(N-N), 679(C-S),; 1HNMR(DMSO)δppm; 9.54(s,1H, OH), 6.86-7.92(m, 9H, Ar-H), 8.26(S, 1H, N=CH-); MS (m/z) : 281(M+).

For compound **7b** Melting point 175-178°C, yield-62%, R_f – 0.38, ; IR (KBr)cm^{-1} : 2910C-H), 1660 (=N), 1567(C=C), 1520(Ar-NO_2),846(N-N), 690(C-S),; 1HNMR(DMSO)δppm; 6.84-8.21(m, 9H, Ar-H), 8.24(S, 1H, N=CH-); MS (m/z) : 310(M+).

For compound **7c** Melting point 168-172°C, yield-66%, R_f – 0.64; IR (KBr)cm^{-1} : 2915C-H), 1658 (C=N), 1566(C=C), 843(N-N), 720(Ar-Cl), 688(C-S); 1HNMR(DMSO)δppm; 1HNMR(DMSO)δppm; 6.88-8.12(m, 9H, Ar-H), 8.17(S, 1H, N=CH-); MS (m/z) : 299(M+).

For compound **7d** Melting point 212°C; yield-70%, R_f – 0.43,,IR (KBr)cm^{-1} : 3559(Ar.OH), 3298(N-H), 2911C-H), 1713(C=O),1652 (C=N), 1563(C=C), 1326(Ar. C-N), 844(N-N), 682(C-S),; 1HNMR(DMSO)δppm; 9.74(s,1H, OH),9.66(s,1H, NH) 6.72-7.8.08(m, 9H, Ar-H), 8.24(S, 1H, N=CH-)2.01 (s,3H, CH_3); MS (m/z) : 338(M+).

For compound **7e** Melting point 208°C; yield-66%, R_f – 0.68, ; IR (KBr)cm^{-1} : 3304(N-H), 2913C-H), 1718(C=O), 1654 (C=N), 1324(Ar. C-N), 1565(C=C), 846(N-N), 689(C-S), 1510(Ar-NO_2); 1HNMR(DMSO)δppm; 1HNMR(DMSO)δppm ; 10.62(s,1H, NH) 6.74-8.19(m, 9H, Ar-H), 8.62(S, 1H, N=CH-)2.03 (s,3H, CH_3); MS (m/z) : 366(M+).

For compound **7f** Melting point 211°C; yield-53%, R_f – 0.54; IR (KBr)cm^{-1} : 2918C-H), 1660 (=N), 3300(N-H),1328(Ar. C-N), 1715(C=O), 1562(C=C), 849(N-N), 720(Ar-Cl), 686(C-S),; 1HNMR(DMSO)δppm: 1HNMR(DMSO)δppm ; 9.76(s,1H, NH) 6.71-7.8.18(m, 9H, Ar-H), 8.27(S, 1H, N=CH-)2.11 (s,3H, CH_3); MS (m/z) : 356(M+).

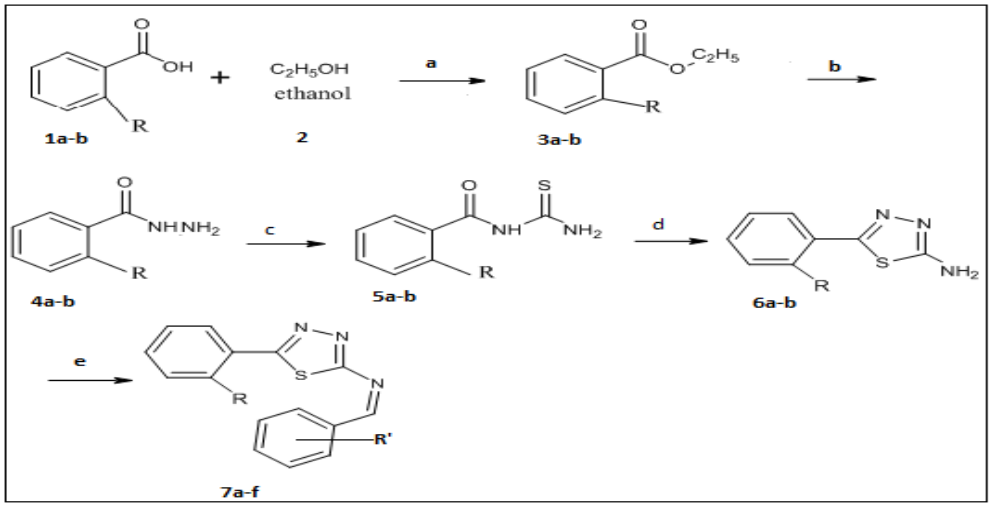


Fig. 1: Synthetic route of substituted (Z)-2-(((5-phenyl-1,3,4-thiadiazol-2-yl)imino)methyl) derivatives (7a-f).

R in compounds 7a-c was hydrogen and in compounds 7d-f was acetamide; R' in compounds 7a and 7d was hydroxyl group, 7b and 7e was nitro group, 7c and 7f was chloro group. a) Conc. Sulphuric acid, reflux for 10-12 hrs b) Hydrazine, methanol, reflux for 8-10 hrs c) Potassium thiocyanide, methanol, reflux for 5-6 hrs d) Conc. Sulphuric acid, reflux for 5-6 hrs e) Salicyldehyde/p-Nitrobenzaldehyde/p-Chlorobenzaldehyde, methanol, reflux for 10-12 hrs.

Antimicrobial activity study[14,15]**:**

The antibacterial activity of synthesized compounds (7a-f) were determined by using agar diffusion method and tube dilution method against one gram positive bacteria namely *Staphylococcus aureus*and three gram negative bacteria namely *Pseudomonas mirabilis and Pseudomonas aeruginosa* and *E.coli*. The antifungal activity of synthesized compounds (7a-f) were determined against two fungus *Aspergillusflavus* and *Aspergillus niger*. The compounds (7a-f) were dissolved in sterile DMF at a concentration µg/ml; uniform holes(6mm) was made in the agar plate by the help of a sterile borer and 0.2ml of the solution was filled in the holeof the agar plate seeded with the test micro-organism and a blank with sterile DMF was carried out as a negative control. Ampicillintrihydrate was used as standard for antimicrobial activity and Fluconazole for antifungal activity. After incubator for 24 hrs at 37°C, the diameter of the inhibitory zone around the hole was measured. Minimum inhibitory concentration (MIC) was defined as the lowest concentration of the test compounds which did not induce visible growth in comparison with a blank experiment after incubation. All experiments were run intriplicate. Tube dilution method: The compounds (7a-f) were dissolved in sterile DMF at a concentration 5,15, 25,75 µg/ml. In test tube 8 ml culture media, 1ml of broth culture of test organisms and 1 ml of test solution was taken under aseptic condition. Then plugged and incubated at 37°C for 24 hrs. Ampicillintrihydrate was used as standard for antimicrobial activity and Fluconazole for antifungal activity. The test tubes were examined for visible turbitdity.

RESULTS AND DISCUSSION

The 2-amino-substituted 5-phenyl-1,3,4-Thiadiazole derivatives(7a-7f) were obtained in good yields. The structures were confirmed on the basis of spectral data.Antibacterial and antifungal activities of all synthesized compounds were determined by the agar diffusion method and also with turbidity method. Results are exhibited in Table 1,2& 3 and its graphical representation are exhibited in Figure 1 and 2. Diameters of inhibitory zones in mm of antibacterial and antifungal are represented in Table 1 and 3. Compounds 7e and 7f exhibited good antibacterial activity against gram positive bacteria and Compounds 7e and 7f also exhibited good antibacterial activity against different gram negative bacterial strains. The activity is may be due to that the both compounds 7e and 7f contains the acetamide group and electron withdrawing group's i.e nitro and chlorine group. Compounds 7c and 7f exhibited moderate antifungal activity against different fungal strains. Other compounds 7a, 7b, and 7d showed moderate antimicrobial activity.

Table 1. Antibacterial(by agar diffusion method)

Compounds	7a	7b	7c	7d	7e	7f	AmpicillinTrihydrate
S. aureus	6.8	6.8	8	5	9	8.5	15
% inhibition	45.3%	45.3%	53.3%	33.3%	60%	56.7%	100%
E. coli	5.5	9	8.2	6.5	7.2	8	13
% inhibition	42%	69%	63.1%	50%	55.4%	61.5%	100%
P. mirabilis	5.3	7.8	8	4.5	9	9	13.2
% inhibition	40.2%	56.8%	60.6%	34.1%	68.2%	68.24%	100%
P. aeruginosa	8	5.5	5	8	8	7	13.5
% inhibition	59.3%	37%	37%	59.3%	59.3%	51.9%	100%

Data presented in mean ±SD (n=3), concentration of derivatives 25µg/ml and concentration of AmpicillinTrihydrate = 25µg/ml. In comparison to control, % inhibition by the synthesized compounds is significant and potent but not showing better activity than the standard drug used in this experiment.

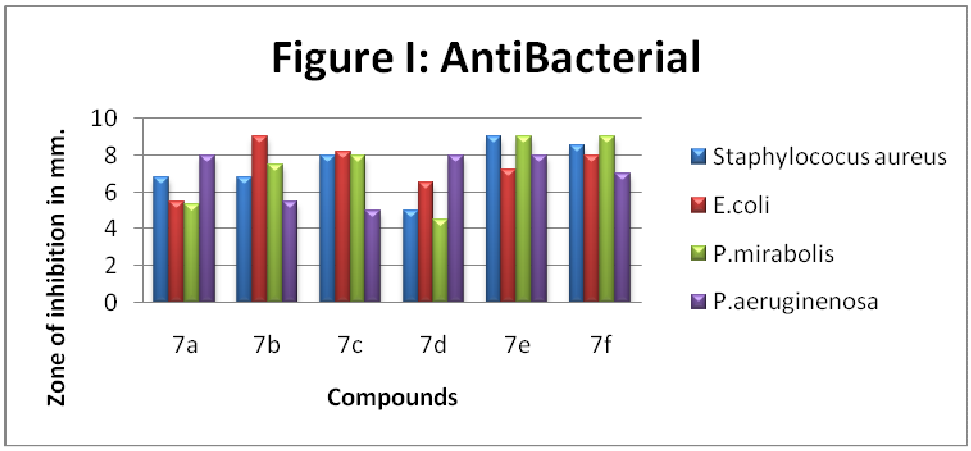


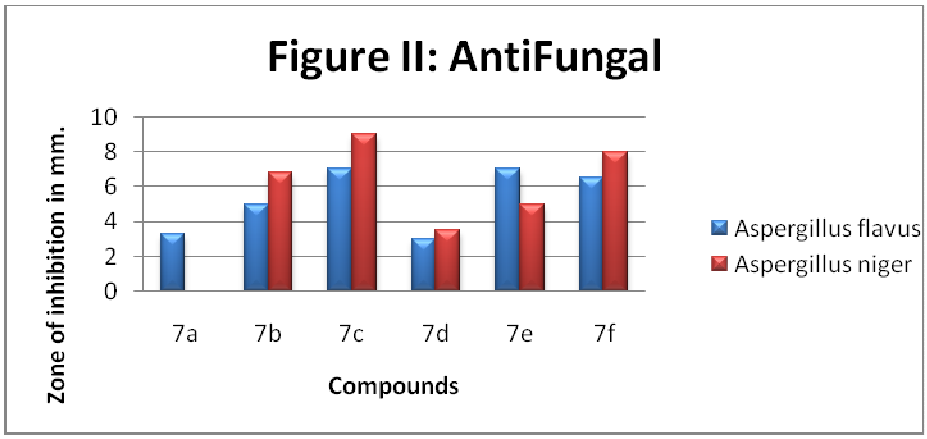
Table 2. Antibacterial (by turbidity method)

Compounds	7a				7b				7c				7d				7e				7f			
Concentration	5	15	25	75	5	15	25	75	5	15	25	75	5	15	25	75	5	15	25	75	5	15	25	75
S. areus	+	+	-	-	+	+	-	-	+	-	-	-	+	+	-	-	+	-	-	-	+	-	-	-
E. coli	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	-	-	-
P.mirabilis	+	+	-	-	+	+	-	-	+	-	-	-	+	+	-	-	+	+	-	-	+	-	-	-
P. aeruginosa	+	+	-	-	+	-	-	-	+	+	-	-	+	+	-	-	+	-	-	-	+	+	-	-

Table 3. Antifungal (by agar diffusion method)

Compounds	7a	7b	7c	7d	7e	7f	Fluconazole
A. flav	3.3	5	7	4.3	7	6.5	17
% inhibition	19%	29%	41%	25%	41%	38%	100%
A. nig	-	6.8	9	3.5	5	8	19
% inhibition	0%	36%	47.4%	18%	26%	42%	100%

Data presented in mean ±SD (n=3), concentration of derivatives 75µg/ml and concentration of Fluconazole = 25µg/ml. In comparison to control, % inhibition by the synthesized compounds is significant and potent but not showing better activity than the standard drug used in this experiment.



CONCLUSION

A series of 2-amino-substituted-5-phenyl-1,3,4-Thiadiazole derivatives was synthesized with good yields and their structures were elucidated by spectral data. Compounds 7e and 7f exhibited good antibacterial activity which may be due the presence of acetamide and electron withdrawing groups. Compounds 7c and 7f showed moderate antifungal activity. Compounds 7a, 7b and 7d showed moderate to low antimicrobial activity as compared with the standard AmpicillinTrihydrate for antibacterial and Fluconazole for antifungal activity.

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