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Synthesis, Characterization and Antimicrobial Studies of Some Novel Thiadiazoles derived from [1,2,4]Triazoles

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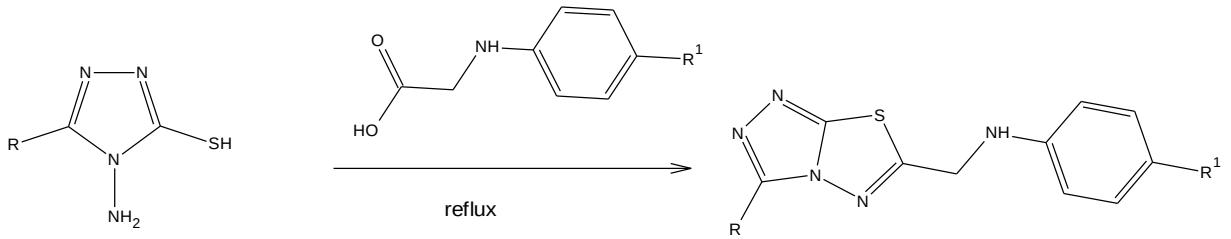
ABSTRACT

*During the present investigation, a new series of 3,6-disubstituted[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole derivatives (**3**) were synthesized by refluxing a mixture of 3-substituted-4-amino-5-mercapto-1,2,4-triazoles with substituted anilinoacetic acids in presence of phosphoryl chloride in good yield. The newly synthesized compounds were confirmed on the basis of elemental analyses, IR, ^{1}H NMR and Mass spectral data. All compounds were screened for their antibacterial and anti-fungal activity. Among the synthesized compounds **(3c), (3f), (3g), (3j)** and **(3m)** exhibited good antibacterial activity and antifungal activity.*

Graphical Abstract

A new series of novel thiadiazoles derived from [1, 2, 3] -triazoles were synthesized, characterized by spectral and analytical data and screened for antibacterial and antifungal activities. Compounds containing chlorine and nitro groups exhibited significant activity.



R = H, CH_3, C_2H_5, C_6H_5,, R^1 = H, CH_3, Cl, NO_2

Keywords: [1,2,4]-triazoles, [1,3,4]-thiadiazoles, [1,2,4]-triazolo-[3,4-b][1,3,4]-thiadiazoles, antibacterial, antifungal.

INTRODUCTION

Heterocyclic compounds especially those containing sulphur and nitrogen atoms possess a wide variety of biological activities. Triazole derivatives have occupied a unique position in heterocyclic chemistry due to their antimicrobial activities [1,2]. In the recent years 1,2,4-triazoles have captured the attention owing to

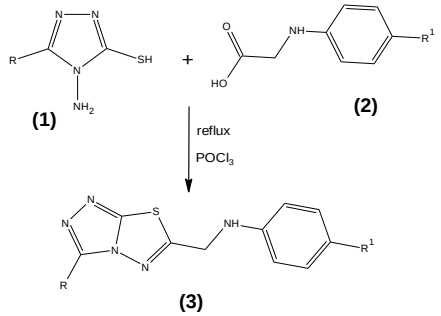
their effective application as drugs in the treatment of various diseases. 1,2,4-Triazole derivatives are known to possess broad spectrum of biological activities such as antimicrobial and anticancer[3], antitubercular[4], analgesic and anti-inflammatory[5] and antiviral[6,7]. Mainly 1,2,4-triazole derivatives are successfully marketed as broad spectrum antifungal drugs such as fluconazole, voriconazole and itraconozole. Moreover several other 1,2,4-triazole derivatives used as drugs such as Ribaverine (antiviral agent), Rizatriptan (antimigraine agent) and Alprazolam (anxiolytic agent). 1,3,4-Thiadiazole is a versatile moiety that exhibits wide variety of biological activities [8,9]. The earliest use of thiadiazoles was in pharmaceutical area as antibacterial with properties similar to those of sulphonamide drugs. Later they were found to possess diverse biological activities like anticancer[10], antiproliferative[11], antioxidant[12] and antidepressant[13]. The extensive application of these two scaffolds in drug designing has made us to synthesis some new molecules comprising these two units. Prompted by these observations and as a continuation of our work on the synthesis of biologically active nitrogen and sulfur containing heterocycles[14-16], we report herein the synthesis of new series of thiadiazoles derived from 1,2,4 triazoles and their antibacterial and antifungal activity studies.

MATERIALS AND METHODS

Chemistry: The melting points of the newly synthesized compounds were determined in open capillaries and are uncorrected. The IR spectra were recorded on a NICOLET iZ 10 FT IR spectrophotometer. The ^{1}H NMR spectra were recorded on a VNMRS-400 "Agilent-NMR" using $DMSOd_6$ as solvent and TMS as an internal standard. All chemical shift values are expressed in δ scale downfield from TMS and proton signals are indicated as s=singlet, d=doublet, t=triplet, m=multiplet. Mass spectra of compounds were recorded on water mass spectrometer model Synapt.g2 USA operating at 70eV . The purity of all compounds was confirmed by TLC.

3-Alkyl-4-amino-5-mercapto-1,2,4-triazoles were prepared by refluxing a mixture of thiocarbohydrazide and suitable carboxylic acid following the literature method [17]. 3-Phenyl-4-amino-5-mercapto-1,2,4-triazole was prepared according to the method[18] starting form potassium salt of bezoylhydrazine with carbon disulphide in alcoholic KOH. Substituted anilinoacetic acids were prepared by refluxing substituted aniline with ethylchloroacetate in presence of sodium hydroxide.

General method for the preparation of 3,6-disubstituted[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (3): A mixture of anilinoacetic acid **(2)** (0.01 mol), 3-substituted-4-amino-5-mercapto-1,2,4-triazole **(1)** (0.01 mol) and phosphoryl chloride (10 mL) was heated under reflux for 6 to 8 h. The reaction mixture was cooled and allowed to stand at room temperature for 2 h. It was then poured into crushed ice. The solid mass formed was filtered, washed with sodium bicarbonate solution, then with water, dried and recrystallized from ethanol/dioxan mixture. The yield, melting point and other characterization data of newly synthesized compounds (**3**) are given in Table-1.



Scheme 1 R = H, CH_3, C_2H_5, C_6H_5; R^1 = H, CH_3, Cl, NO_2

Table: 1. Characterization data of preparation of 3,6-disubstituted[1,2,4]triazolo[3,4-*b*][1,3,4] thiadiazole (3a-m)

Comp No.	R	R^1	Yield (%) M.P(^OC)	Molecular formula	Color & Crystal nature	Analysis (%) found (calculated)		
						C	H	N
(3a)	H	H	63 155-158	$C_{10}H_9N_5S$	Brown color powder	51.60 (51.93)	3.88 (3.92)	30.33 (30.28)
(3b)	H	CH_3	64 158-160	$C_{11}H_{11}N_5S$	Brown color powder	53.79 (53.86)	4.50 (4.52)	28.54 (28.55)
(3c)	H	Cl	62 156-159	$C_{10}H_8N_5SCl$	Brown color powder	45.19 (45.20)	3.00 (3.03)	26.33 (26.36)
(3d)	CH_3	H	68 180-185	$C_{11}H_{11}N_5S$	Brown color powder	53.84 (53.86)	4.44 (4.52)	28.52 (28.55)
(3e)	CH_3	CH_3	69 165-170	$C_{12}H_{13}N_5S$	Light brown color powder	55.55 (55.58)	5.01 (5.05)	27.00 (27.01)
(3f)	CH_3	Cl	73 120-125	$C_{11}H_{10}N_5SCl$	Light brown color powder	47.22 (47.23)	3.55 (3.60)	25.01 (25.03)
(3g)	CH_3	NO_2	69 175-179	$C_{11}H_{10}N_6O_2S$	Yellow color powder	45.46 (45.51)	3.43 (3.47)	28.88 (28.95)
(3h)	C_2H_5	H	66 155-159	$C_{12}H_{13}N_5S$	Brown color powder	55.55 (55.58)	5.00 (5.05)	27.06 (27.01)
(3i)	C_2H_5	CH_3	67 161-164	$C_{13}H_{15}N_5S$	Dark brown color crystals	57.11 (57.12)	5.51 (5.53)	25.59 (25.62)
(3j)	C_2H_5	Cl	65 155-158	$C_{12}H_{12}N_5SCl$	Dark brown color powder	49.03 (49.06)	4.00 (4.12)	23.81 (23.84)
(3k)	C_6H_5	H	67 161-165	$C_{16}H_{13}N_5S$	Dark brown color powder	62.49 (62.52)	4.25 (4.26)	22.77 (22.78)
(3l)	C_6H_5	CH_3	70 163-165	$C_{17}H_{15}N_5S$	Dark brown color powder	63.51 (63.53)	4.69 (4.70)	21.77 (21.79)
(3m)	C_6H_5	Cl	71 162-165	$C_{16}H_{12}N_5SCl$	Dark brown color powder	56.20 (56.22)	3.52 (3.54)	20.45 (20.49)

Antimicrobial Activity: The newly synthesized compounds (**3a-m**) (25µg mL^{-1}) were screened for their antibacterial activity *in-vitro* against both Gram-positive and Gram-negative bacteria. *Staphylococcus aureus*(**NCIM 2794**), *Bacillius subtilis* (**NCIM 2708**), *Escherichia coli*(**NCIM 2575**) and *Pseudomonas aeruginosa*(**NCIM 2053**) were the microorganisms employed. Sulfa-methoxazole was used as standard. The antifungal activity was screened *in-vitro* against fungi *Aspergillus niger* (**NCIM 619**) *Candida albicans* (**NCIM 3466**) and *Rhizopus stolonifer* (**NCIM 1139**). *Griseofulvin* was the standard. The dimethylformamide was used as solvent control. The culture media was nutrient agar and the method employed was cup-plate method [19]. The diameter of zones of inhibition was measured in mm.

RESULTS AND DISCUSSION

Chemistry: The structures of triazolothiadiazole derivatives (**3a-m**) were established on the basis of elemental analyses, IR, ^{1}H NMR and mass spectral data. The results of elemental analysis of synthesized compounds were in agreement with the theoretical values within the limits of experimental error.

In the IR spectra of triazolothiadiazoles (**3a-m**), the absorption bands corresponding to the NH_2 stretching frequency of the starting triazole and OH stretching frequency of carboxylic group of anilino acetic acid were absent. This confirmed the involvement of these groups in the ring formation. The IR spectrum of (**3f**) showed absorption bands in the region around 1600cm^{-1} characteristic of C=N stretching. Band due to NH stretching was appeared as broad peak at 3300cm^{-1}. Further in the ^{1}H-NMR spectrum of compound (**3f**) (solvent $DMSOd_6$) shows a singlet at δ, 2.585 integrating for three protons. The peak at δ, 4.641 integrating for two protons confirms the presence of N-CH_2 group. The signal due to NH proton appeared

as singlet at δ, 6.874 integrating for one proton. The aromatic protons of p-chlorophenyl group appeared as two doublets centered at δ, 6.659 and δ, 7.119 integrating for two protons each. Further evidence in support of the assigned structure for these compounds was obtained by recording their mass spectra. In the mass spectrum of compound (**3f**) the M^+ peak was observed at m/z, 279 consistent with the molecular formula $C_{11}H_{10}N_5SCl$. The chlorine isotopic peak was observed at m/z, 281 in ration 3:1 confirms the formations of triazolothiadiazole. The spectral characteristics of remaining compounds are as follows.

6-(Anilinomethyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (3a): IR (KBr) *v* cm^{-1}: 3281cm^{-1}(N-H. Stretch); 2915cm^{-1}(C-H stretch); 1598cm^{-1}(C=N stretch)): ^{1}H-NMR (400MHz); Solvent DMSO-d_6: δ, 4.640(s, 2H, N-CH_2); δ, 6.674 (s, 1H, NH). δ, 7.123-7.158(m, 5H, ArH); 7.813(s, 1H, triazole-3H): MS, m/z; 231(M^+).

6-(4-methylanilinomethyl- [1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (3b): IR (KBr) *v* cm^{-1}: 3280cm^{-1}(N-H. Stretch); 2906cm^{-1}(C-H stretch); 1586cm^{-1}(C=N stretch)): ^{1}H-NMR (400MHz); Solvent DMSO-d_6: δ, 2.303(s, 3H, CH_3); δ, 4.630(s, 2H, N-CH_2); δ, 6.658(d, 2H, ortho protons of p-tolyl); δ, 7.023(d, 2H, meta protons of p-tolyl); δ, 6.682 (s, 1H, NH). MS, m/z; 245(M^+).

6-(4-Chloroanilinomethyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (3c): IR (KBr) *v* cm^{-1}: 3271cm^{-1}(N-H. Stretch); 2926cm^{-1}(C-H stretch); 1592cm^{-1}(C=N stretch)): ^{1}H-NMR (400MHz); Solvent DMSO-d_6: δ, δ, 4.640(s, 2H, N-CH_2); δ, 6.680(d, 2H, meta protons of p-chlorophenyl); δ, 7.110(d, 2H, ortho protons of p-chlorophenyl); δ, 6.670(s, 1H, NH). 7.813(s, 1H, triazole-3H): MS, m/z; 265(M^+). m/z; 267(M+2).

6-(Anilinomethyl)-3-methyl[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (3d): IR (KBr) *v* cm^{-1}: 3283cm^{-1}(N-H. Stretch); 2912cm^{-1}(C-H stretch); 1585cm^{-1}(C=N stretch)): ^{1}H-NMR (400MHz); Solvent DMSO-d_6: δ, 2.320(s, 3H, CH_3); δ, 4.640(s, 2H, N-CH_2); δ, 6.674 (s, 1H, NH); δ, 7.110- 7.123(m, 5H, ArH); MS, m/z; 245(M^+).

6-(4-methylanilinomethyl)-3-methyl[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (3e): IR (KBr) *v* cm^{-1}: 3280cm^{-1}(N-H. Stretch); 2910cm^{-1}(C-H stretch); 1590cm^{-1}(C=N stretch)): ^{1}H-NMR (400MHz); Solvent DMSO-d_6: δ, 2.213(s, 3H, CH_3); 2.984(s, 3H, CH_3); δ, 4.640(s, 2H, N-CH_2); δ, 6.688(d, 2H, ortho protons of p-tolyl); δ, 7.113(d, 2H, meta protons of p-tolyl); δ, 6.674 (s, 1H, NH). MS, m/z; 259(M^+).

6-(4-Nitroanilinomethyl)- 3-methyl [1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (3g): IR (KBr) *v* cm^{-1}: 3275cm^{-1}(N-H. Stretch); 2914cm^{-1}(C-H stretch); 1592cm^{-1}(C=N stretch); 1560cm^{-1}(N=O stretch)): ^{1}H-NMR (400MHz); Solvent DMSO-d_6: δ, 2.213(s, 3H, CH_3); δ, 4.644(s, 2H, N-CH_2); δ, 6.650(d, 2H, meta protons of p-nitrophenyl); δ, 7.120(d, 2H, ortho protons of p-nitroophenyl); δ, 6.670 (s, 1H, NH). MS, m/z; 290(M^+).

6-(Anilinomethyl)- 3-ethyl [1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (3h): IR (KBr) *v* cm^{-1}: 3281cm^{-1}(N-H. Stretch); 2926cm^{-1}(C-H stretch); 1598cm^{-1}(C=N stretch)): ^{1}H-NMR (400MHz); Solvent DMSO-d_6: δ, 1.413(t, 3H, CH_3); 2.986(q, 2H, CH_2); δ, 4.646(s, 2H, N-CH_2); δ, 7.120- 7.223(m, 5H, ArH); MS, m/z; 259(M^+).

6-(4-Nitroanilinomethyl)-3-ethyl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (3i): IR (KBr) *v* cm^{-1}: 3281cm^{-1}(N-H. Stretch); 2916cm^{-1}(C-H stretch); 1596cm^{-1}(C=N stretch)): ^{1}H-NMR (400MHz); Solvent DMSO-d_6: δ, 1.313(t, 3H, CH_3); 2.984(q, 2H, CH_2); δ, 4.640(s, 2H, N-CH_2); δ, 6.688(d, 2H, ortho protons of p-tolyl); δ, 7.113(d, 2H, meta protons of p-tolyl); δ, 6.674 (s, 1H, NH). MS, m/z; 273(M^+).

6-(4-Chloroanilinomethyl)-3-ethyl[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (3j): IR (KBr) *v* cm^{-1}: 3281cm^{-1}(N-H. Stretch); 2916cm^{-1}(C-H stretch); 1596cm^{-1}(C=N stretch)): ^{1}H-NMR (400MHz); Solvent DMSO-d_6: δ, 1.313(t, 3H, CH_3); 2.984(q, 2H, CH_2); δ, 4.640(s, 2H, N-CH_2); δ, 6.655(d, 2H, meta protons

of p-chlorophenyl); δ, 7.123(d, 2H, ortho protons of p-chlorophenyl); δ, 6.674 (s, 1H, NH). MS, m/z; 293(M^+). m/z; 295(M+2).

6-(Anilinomethyl)-3-phenyl[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (3k): IR (KBr) *v* cm^{-1}: 3284cm^{-1} (N-H. Stretch); 2926cm^{-1}(C-H stretch); 1598cm^{-1}(C=N stretch)): ^{1}H-NMR (400MHz); Solvent DMSO-d_6: δ, 4.640(s, 2H, N-CH_2); δ, 6.650- δ, 7.123(m, 10H, ArH); MS, m/z; 307 M^+).

6-(4-Methylanilinomethyl)-3-ethyl[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (3l): IR (KBr) *v* cm^{-1}: 3281cm^{-1}(N-H. Stretch); 2922cm^{-1}(C-H stretch); 1586cm^{-1}(C=N stretch)): ^{1}H-NMR (400MHz); Solvent DMSO-d_6: δ, 2.413(s, 3H, CH_3); δ, 6.688(d, 2H, ortho protons of p-tolyl); δ, 7.113(d, 2H, meta protons of p-tolyl); δ, 6.674 (s, 1H, NH). 7.204- δ, 7.223(m, 5H, ArH); MS, m/z; 321(M^+).

6-(4-Chloroanilinomethyl)-3-phenyl[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (3m): IR (KBr) *v* cm^{-1}: 3317cm^{-1}(N-H. Stretch); 2917cm^{-1}(C-H stretch); 1597cm^{-1}(C=N stretch):^{1}H-NMR (400MHz); Solvent DMSO-d_6: 2.984(q, 2H, CH_2); δ, 4.640(s, 2H, N-CH_2); δ, 6.655(d, 2H, meta protons of p-chlorophenyl); δ, 7.123(d, 2H, ortho protons of p-chlorophenyl); 7.204- δ, 7.223(m, 5H, ArH); δ, 6.674 (s, 1H, NH). MS, m/z; 341(M^+). m/z; 343(M+2).

Antimicrobial activity: Results of antibacterial and antifungal screening study are depicted in table **2**. The compounds **(3c), (3f), (3g), (3j)** and **(3m)** showed maximum zone of inhibition against the tested organism compared with that of standard drug. The remaining compounds showed good to moderate activity against both gram positive and gram negative bacterial stains. The results of antimicrobial activity study shows that compounds containing electron withdrawing nitro and chloro groups show maximum inhibition. Structure-activity relationship for the observed activity and substitution in the aryl ring can be done. From structure activity relationship results it is clear that, chlorine and nitro group are responsible for greater antibacterial and antifungal activity.

Table-2: Antibacterial and antifungal activity of Triazolothiadiazoles **(3a-m)**

Comp. No	Antibacterial Activities				Antifungal Activities		
	Diameter of zone of inhibition (in mm)				Diameter of zone of inhibition (in mm)		
	Ps. Aer.	*B. subt.*	*S. aur.*	*E. coli*	*A. niger*	*C. albicans*	*R. stolonifer*
(3a)	10	10	10	08	12	10	08
(3b)	10	10	12	05	09	12	08
(3b)	11	12	11	10	14	12	10
(3c)	14	15	13	11	15	14	15
(3d)	12	12	11	10	09	06	06
(3e)	10	11	10	09	10	09	12
(3f)	14	14	14	11	14	15	12
(3g)	14	15	14	15	15	14	15
(3h)	12	14	10	07	08	13	11
(3i)	09	12	13	06	05	08	12
(3j)	14	15	15	14	12	15	14
(3k)	08	10	11	10	12	11	10
(3l)	10	12	12	07	05	08	10
(3m)	15	15	13	12	14	12	14
Control	-	-	-	-	-	-	-
Sulfa-methoxazole	14	15	14	12	-	-	-
Griseofulvin	-	-	-	-	15	15	16

Index for antimicrobial activities:
Diameter of the cup: 5mm
Amount of the sample used: 25µg cup^{-1}
Control: Dimethyl formamide
Standard drug used: Sulfa-methoxazole* and Griseofulvin#

Abbreviation used:
P. aer: Pseudomonas aeruginosa
B. subt.: Bacillus subtilis
S. *aur.: Staphylococcus aureus*
E. coli.: Escherichia coli
A. niger: Asperagillus niger
C. albicans: Candida albicans
R. stolonifer: Rhizopus stoloinifer

APPLICATIONS

The syntheses of derivatives of triazolothiadiazoles that have been reported in this give different approaches to the challenge of preparing these bioactive products and allow the synthesis of many novel chemical derivatives. These derivatives have vast range of biological activities.

CONCLUSIONS

We have synthesized some novel Triazolothiadiazole derivatives in good yield and evaluated them for their *in vitro* antibacterial and antifungal activities. The compounds **(3c), (3f), (3g), (3j)** and **(3m)** showed maximum zone of inhibition against the tested organism compared with that of standard drug. Thus, the above compounds can be considered as lead compounds for enhancing their activity and development of more potent antibacterial and antifungal agents.

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