INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

SYNTHESIS AND BIOLOGICAL EVALUATION OF CONDENSED 2,6-DISUBSTITUTED-[1,2,4]TRIAZOLO][5, 1-b][1,3,4] THIADIAZOL-5-AMINE DERIVATIVES AS POSSIBLE ANTIMICROBIAL AND ANALGESIC AGENTS

Wakale VS[1*], Nandal DH[2] and Vachane Patil RR[3]

[1]Research Scholar, Pravara Institutes of Medical Sciences (DU), Loni-413 736, Ahmednagar, Maharashtra, India.
[2]Department of Pharmacology, Rural Medical College, PIMS (DU), Loni-413 736, Ahmednagar, Maharashtra, India.
[3]Department of Pharmacy, VJSMs Institute of Pharmacy for Women, Ale-412 411, Tal- Junnar, Dist- Pune, Maharashtra, India.

ABSTRACT

2,6-disubstituted-[1,2,4]triazolo][5,1-b][1,3,4]thiadiazol-5-amine derivatives were synthesized by reacting aryl carboxylic acid with thiosemicarbazide to give 2-amino-5-aryl-1,3,4-thiadiazole (1), which were reacted with substituted aldehydes to yield schiff bases 5-aryl 1,3,4-thiadiazole (2). The resulted thiadiazoles were converted to 2,6-disubstituted triazolo-thiadiazoles by reacting hydrazine hydrate in presence of anhydrous $FeCl_3.6H_2O$. The structures of these newly synthesized compounds were confirmed on the basis of Elemental analysis, IR, ^{1}H-NMR and Mass spectra. All compounds were screened for antimicrobial and analgesic activities. The antimicrobial activity was determined by cup plate method and analgesic activity was determined by hot plate method. Some of the tested compounds showed significant activity.

Keywords: Triazolo-thiadiazole, Antibacterial, Antifungal, Analgesic activity.

INTRODUCTION

Infectious diseases are the leading cause of death worldwide. In recent years a rapid increase in the emergence of microbes that are resistant to conventionally used antibiotics has been observed[1]. Now a day's research is concentrated on the introduction of new and safe therapeutic agents of clinical importance. A large number of N-bridged heterocycles derived from 1,2,4-triazoles are important pharmacotherapeutic agents and a significant amount of research has been directed towards this class of compounds. The combination of two or more biologically active heterocyclic rings, either in condensed form or coupled form, results in reinforcement of biological activity of such compounds by many folds. Moreover, 1,2,4-triazole fused with 1,3,4-thiadiazole ring system showed wide spread attention due to diverse applications, such as antimicrobial[2-3], antitubercular[4], anti-inflammatory [5], analgesic[6], antitumer[7], anticonvulsant[8], antioxidant[9], anticancer[10] activities. As a continuation of present study to explore potent biological compounds, we have synthesized 2, 6-disubstituted-[1,2,4]triazolo][5,1-b][1,3,4]thiadiazol-5-amine and evaluated them for their antimicrobial and analgesic activities.

MATERIAL AND METHODS

Experimental

Melting points were determined in open capillary method and are uncorrected. Purity of the compounds was checked on Silica Gel TLC plates. IR spectra were recorded on Thermo Nicolet IR 200 spectrophotometer using KBr disc method. ^{1}H NMR spectra were recorded on BRUKER AVANCE II 400 NMR Spectrometer, in DMSO-d_6 solvents using

TMS as an internal standard. Elemental analysis was recorded on Perkin-Elmer Model 2400 elemental analyzer. Mass spectra were determined on LC-MS Spectrometer Model Q-ToF Micro Waters.

Preparation of 2-amino-5-aryl-1,3,4-thiadizole (1)[11]

A mixture of thiosemicarbazide (0.1mol), aryl carboxylic acid (0.1mol) and conc. sulphuric acid (5 ml) dissolved in 50 ml of ethanol & refluxed for 2 h & poured into crushed ice. The solid separated out was filtered, washed with water and recrystallized from ethanol to give 2-amino-5-aryl-1,3,4-thiadizole.

Preparation of substituted 5-Phenyl-N-(1Z)-Phenyl methylene 1,3,4-thiadiazole-2-amine (2)[12]

Stir a mix of **1** (0.01mol), 18 ml water, 2.4 ml conc. ammonia and add (0.01mol) substituted aldehydes dropwise with stirring over period of 30-60 min. Stir the mixture of further hour. Stir the mixture for further hour, collect the solid by suction filtration and wash it with water, recrystallized from ethanol.

General procedure for preparation of 2,6-diphenyl-[1,2,4]triazolo[5,1-b][1,3,4]thiadiazol-5(7aH)-amine (R_1)[13]

Mixture of imine **2** (0.01 mol) & $FeCl_3.6H_2O$ (0.02 mol) & 0.01 mol Hydrazine Hydrate was ground by morter & pestal at room temperature. After complete conversion as indicated by TLC. The reaction mixture was digested was water. The resultant solid was filtered, washed with water & crude material is purified by recrystallization. Similar procedure was followed for preparation R_2-R_{10}.

Spectral data of synthesized compounds (R_1-R_{10})

R_1: 2,6-diphenyl-[1,2,4]triazolo[5,1-b][1,3,4]thiadiazol-5(7aH)-amine

IR (KBr)cm^{-1}: 3365.17 (N-H Str), 3099.06 (C-H Ar Str), 1648.84 (C=N Str), 1597.73 (C=C), 1515.78 (C-N Str), 1152.01 (C-S); ^{1}H NMR (DMSO, 400 MHz) δ (ppm): 2.53 (s, 2H, NH_2), 4.87 (s, 1H, -CH methine), 6.84-7.60 (m, 5H, Ar-H), 7.75-8.16 (m, 5H, Ar-H).

R_2:4-(5-amino-2-phenyl-5,7a-dihydro-[1,2,4]triazolo[5,1-b][1,3,4]thiadiazol-6-yl) phenol

IR (KBr)cm^{-1}: 3413.39 (-OH Str), 3225.00 (N-H Str), 3139.54 (C-H Ar Str), 1686.44 (C=N Str), 1609.21 (C=C), 1529.27 (C-N Str), 1153.22 (C-S); ^{1}H NMR (DMSO, 400 MHz) δ (ppm): 2.55 (s, 2H, NH_2), 5.19 (s, 1H, -CH methine), 5.35 (s, 1H, Ar C-OH), 6.85-7.47 (m, 5H, Ar-H), 7.50-7.98 (m, 4H, Ar-H). MS (ESI) m/z: 310.4 [M^+].

R_3:4-(5-amino-2-phenyl-5,7a-dihydro-[1,2,4]triazolo[5,1-b][1,3,4]thiadiazol-6-yl)-2-methoxy phenol

IR (KBr)cm^{-1}: 3385.39 (-OH Str), 3225.10 (N-H Str), 3092.34 (C-H Ar Str), 1615.24 (C=N Str), 1600.21 (C=C), 1496.49 (C-N Str), 1181.19 (C-S); ^{1}H NMR (DMSO, 400 MHz) δ (ppm): 2.53 (s, 2H, NH_2), 3.80 (d. 3H, CH_3), 5.31 (s, 1H, -CH methine), 5.34 (s, 1H, Ar C-OH), 6.82-7.46 (m, 3H, Ar-H), 7.55-7.89 (m, 4H, Ar-H). MS (ESI) m/z: 340.5 [M^+].

R_4:6-(2-chlorophenyl)-2-Phenyl-[1,2,4]triazolo[5,1-b][1,3,4]thiadiazol-5(7aH)-amine

IR (KBr)cm^{-1}: 3360.27 (N-H Str), 3181.97 (C-H Ar Str), 1649.80 (C=N Str), 1598.70 (C=C), 1514.81 (C-N Str), 1170.58 (C-S), 733.82(C-Cl); ^{1}H NMR (DMSO, 400 MHz) δ (ppm): 2.52 (s, 2H, NH_2), 5.15 (s, 1H, -CH methine), 7.40-7.51 (m, 5H, Ar-H), 7.52-8.11 (m, 4H, Ar-H).

R_5: 6-(furan-2-yl)-2-phenyl-[1,2,4]triazolo[5,1-b][1,3,4]thiadiazol-5(7aH)-amine

IR (KBr)cm^{-1}: 3354.09 (N-H Str), 3023.84 (C-H Ar Str), 1647.15 (C=N Str), 1529.38 (C=C), 1490.70 (C-N Str), 1126.22 (C-S); ^{1}H NMR (DMSO, 400 MHz) δ (ppm): 2.42 (s, 2H, NH_2), 4.70 (s, 1H, -CH methine), 6.72-7.74 (m, 3H, Ar-H), 7.78-8.20 (m, 5H, Ar-H).

R_6:2-(4-chlorophenyl)-6-phenyl-[1,2,4]triazolo[5,1-b][1,3,4]thiadiazol-5(7aH)-amine

IR (KBr)cm^{-1}: 3322.47 (N-H Str), 3072.05 (C-H Ar Str), 1697.05 (C=N Str), 1602.56 (C=C), 1496.49 (C-N Str), 1128.15 (C-S), 809.95 (C-Cl); ^{1}H NMR (DMSO, 400 MHz) δ (ppm): 2.54 (s, 2H, NH_2), 5.31 (s, 1H, -CH methine), 7.44-7.57 (m, 4H, Ar-H), 7.59-8.12 (m, 5H, Ar-H).

R_7:4-(5-amino-2-(4-chlorophenyl)-5,7a-dihydro-[1,2,4]triazolo[5,1-b][1,3,4] thiadiazol-6-yl) phenol

IR (KBr)cm^{-1}: 3401.16 (-OH Str), 3340.10 (N-H Str), 3031.55 (C-H Ar Str), 1657.52 (C=N Str), 1606.41 (C=C), 1514.81 (C-N Str), 1170.58 (C-S), 722.21 (C-Cl); ^{1}H NMR (DMSO, 400 MHz) δ (ppm): 2.55 (s, 2H, NH_2), 5.23 (s, 1H, -CH methine), 5.35 (s, 1H, Ar C-OH), 7.37-7.57 (m, 4H, Ar-H), 7.98-8.30 (m, 4H, Ar-H). MS (ESI) m/z: 344.3 [M^+].

R_8:4-(5-amino-2-(4-chlorophenyl)-5,7a-dihydro-[1,2,4]triazolo[5,1-b][1,3,4] thiadiazol-6-yl)-2-methoxyphenol

IR (KBr)cm^{-1}: 3393.36 (-OH Str), 3288.04 (N-H Str), 3072.05 (C-H Ar Str), 1688.37 (C=N Str), 1593.27 (C=C), 1496.49 (C-N Str),

1128.15 (C-S), 809.95 (C-Cl); ^{1}H NMR (DMSO, 400 MHz) δ (ppm): 2.51 (s, 2H, NH_2), 3.84 (d, 3H, CH_3), 5.27 (s, 1H, -CH methine), 5.38 (s, 1H, Ar C-OH), 6.82-6.96 (m, 3H, Ar-H), 7.52-7.77 (m, 4H, Ar-H). MS (ESI) m/z: 376.5 [M^+].

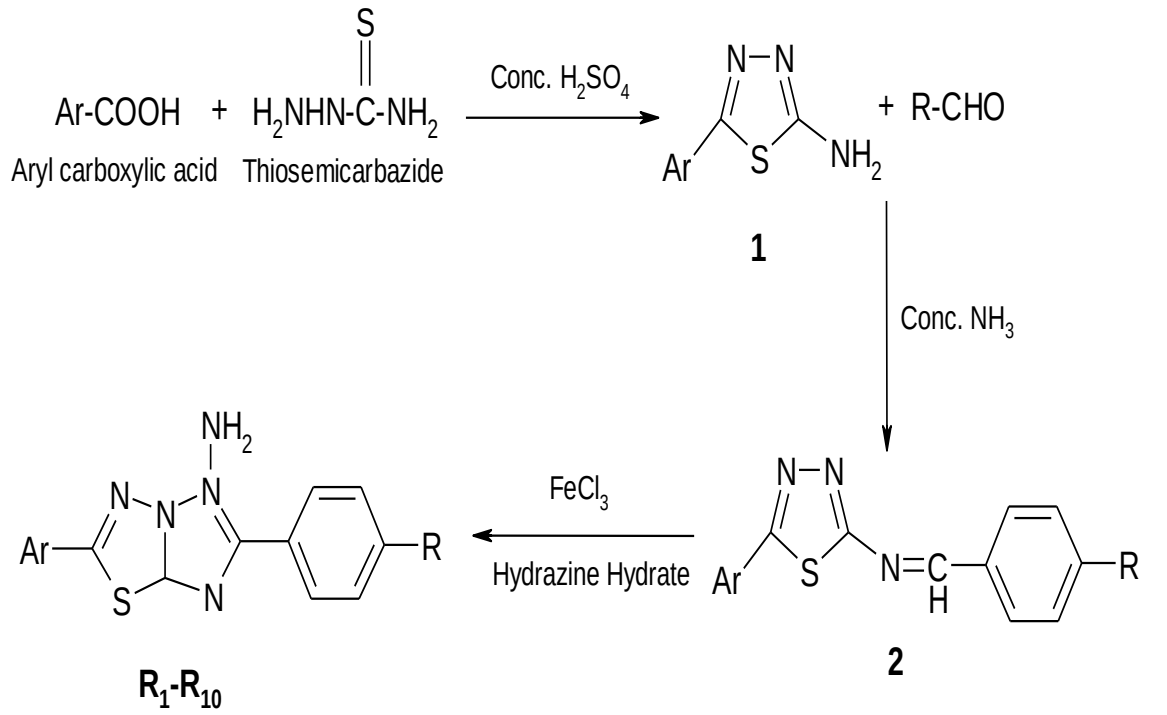
R_9:6-(2-chlorophenyl)-2-(4-chlorophenyl)-[1,2,4]triazolo[5,1-b][1,3,4]thiadiazol-5(7aH)-amine

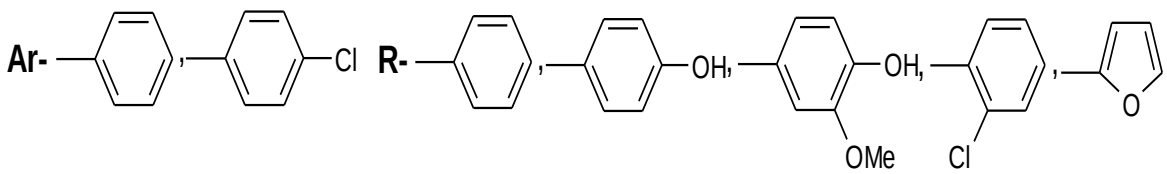
IR (KBr)cm^{-1}: 3359.39 (N-H Str), 3025.76 (C-H Ar Str), 1657.52 (C=N Str), 1598.70 (C=C), 1514.81 (C-N Str), 1167.69 (C-S), 717.39(C-Cl); ^{1}H NMR (DMSO, 400 MHz) δ (ppm): 2.48 (s, 2H, NH_2), 5.31 (s, 1H, -CH methine), 7.40-7.51 (m, 3H, Ar-H), 7.56-8.07 (m, 5H, Ar-H).

R_{10}:2-(4-chlorophenyl)-6-(furan-2-yl)-[1,2,4]triazolo[5,1-b][1,3,4]thiadiazol-5(7aH)-amine

IR (KBr)cm^{-1}: 3264.89 (N-H Str), 3059.51 (C-H Ar Str), 1697.05 (C=N Str), 1599.66 (C=C), 1526.38 (C-N Str), 1176.26 (C-S), 723.17 (C-Cl); ^{1}H NMR (DMSO, 400 MHz) δ (ppm): 2.52 (s, 2H, NH_2), 4.36 (s, 1H, -CH methine), 6.56-7.53 (m, 4H, Ar-H), 7.72-8.21 (m, 3H, Ar-H).

SCHEME





Biological Evaluation

Animals

Albino mice were used for experimental purpose. The animals were housed in hygienic cages under standard conditions of temperature (25±2)^{0}C, relative humidity (45±2)% and (light) 12h: (dark) 12h cycle in registered animal house of Sharadchandra Pawar College of Pharmacy, Otur (Registration no. 1197/po/c/08/CPCSEA). The animals were fed with standard pellet diet and water *ad libitum*. The experimental design and research plan along with animals handling and disposal procedure were approved by Institutional Animal Ethical Committee (IAEC) and IAEC approval number was SPCOP/IAEC/2013-2014/08.

Antimicrobial Activity[14]

The antimicrobial activity of the synthesized compounds was determined by cup-plate method. The organisms selected for antibacterial activity were *Escherichia coli* and *Staphylococcus aureus.* Similarly the antifungal activity was carried out by using *Aspergillus niger* and *Candida albicans.* The concentration of sample compound was 100µg/ml. Norfloxacin and Griseofulvin was used as standard drugs for antibacterial and antifungal activity respectively. Control test with solvents were performed for every assay but showed no inhibition of the microbial growth. Zone of inhibition of tested compounds against various species were shown in table 2.

Analgesic Activity[15]

All the synthesized compounds were screened for analgesic activity by using hot plate method on albino mice and the instrument used for this purpose was Eddy's hot plate. The dose of synthesized compounds administered was 50 mg/kg by oral route using oral feeding tuberculin syringe. The stock suspensions of standard and synthesized compounds were prepared in concentration of 10 mg/ml of 2% *w/v* Carboxy methyl Cellulose (CMC) in distilled water. The control group was treated with vehicle CMC. Ibuprofen was used as standard drug for comparison. The basal reaction time, for jump response, when animals placed on hot plate (maintained at constant temperature of 55±1.0ºC) was observed and reaction time of animals on hot plate at 0.5 h, 1 h and 1.5 h, after administration of the test and standard compounds, was also noted. Percentage inhibitions shown by tested compounds were recorded in table 3.

Statistical analysis

Data were presented as arithmetic Mean±SEM. Statistical analysis was performed by One Way Variance (ANOVA) followed by Dunnett's test. ''*p*'' value of less than 0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

The synthetic pathway is presented in scheme. 2,6-disubstituted-[1,2,4]triazolo][5,1-b][1,3,4]thiadiazol-5-amine derivatives (R_1-R_{10}) have been synthesized. All these compounds were characterized by Elemental analysis, IR, ^{1}H-NMR and Mass spectra. All the synthesized compounds were screened for their antimicrobial activities. Compounds R_3, R_7, R_8 and R_9 have shown promising antibacterial activity, while remaining compounds shown moderate antibacterial activity against *E. coli* and *S. aureus.* Compounds R_1, R_3, R_7 and R_9 have shown potent antifungal activity against *A. niger* and *C. albicans,* remaining compounds shown good activity when compared with standard.

The synthesized compounds were also screened for analgesic activity by hot plate method. The majority of tested compounds showed varying degrees of activity. The highest activity was shown by compounds R_8 and R_9, which produced strong dose dependant inhibition (>50%), while compounds R_3, R_4, R_6 and R_7 were shown moderate inhibition ((40-46%).

CONCLUSION

In conclusion series of 2,6-disubstituted-[1,2,4]triazolo][5,1-b][1,3,4]thiadiazol-5-amine derivatives have been synthesized. All the compounds were obtained in good yield and were tested as potential biological agents. It has been noticed that modifications on triazolo-thidiazoles displayed valuable biological activities, which need to be further investigated to get better agents.

ACKNOWLEDGEMENTS

Authors are grateful to the Principal Dr. G Y Dama, Sharadchandra Pawar College of Pharmacy, Otur for providing necessary facilities to carry out the work. The authors are also grateful to Dr. Pattan S R for their constant encouragement and the director, SAIF, Punjab University, Chandigarh for providing NMR, Mass spectral data.

Table 1: Physical and Analytical Data of Synthesized compounds (R_1- R_{10})

Comp.	Mol. Formula	Mol. Wt.	M.P. ^0C	Rf Value	Yield (%)	Elemental analysis calcd. (found)			
						C	H	N	S
R_1	$C_{15}H_{13}N_5S$	295	189-191	0.58	62	54.63 (54.66)	3.67 (3.70)	21.23 (21.26)	9.72 (9.76)
R_2	$C_{15}H_{13}N_5OS$	311	178-180	0.63	80	52.10 (52.14)	3.50 (3.53)	20.25 (20. 28)	9.27 (9.30)
R_3	$C_{16}H_{15}N_5O_2S$	341	199-201	0.46	55	51.13 (51.16)	3.75 (3.73)	18.63 (18.66)	8.53 (8.50)
R_4	$C_{15}H_{12}ClN_5S$	329	205-207	0.59	63	49.46 (49.49)	3.04 (3.07)	19.23 (19.23)	8.80 (8.83)
R_5	$C_{13}H_{11}N_5OS$	285	167-169	0.41	60	48.83 (48.80)	3.15 (3.18)	21.90 (21.93)	10.03 (10.00)
R_6	$C_{15}H_{12}ClN_5S$	329	172-174	0.53	68	61.00 (61.04)	4.44 (4.47)	23.71 (23.74)	10.86 (10.90)
R_7	$C_{15}H_{12}ClN_5OS$	345	184-186	0.66	76	57.86 (57.83)	4.21 (4.25)	22.49 (22.51)	10.30 (10.34)
R_8	$C_{16}H_{14}ClN_5O_2S$	375	218-220	0.43	72	56.29 (56.29)	4.43 (4.46)	20.51 (20.55)	9.39 (9.42)
R_9	$C_{15}H_{11}Cl_2N_5S$	364	156-158	0.48	59	54.63 (54.66)	3.67 (3.70)	21.23 (21.27)	9.72 (9.76)
R_{10}	$C_{13}H_{10}ClN_5OS$	319	200-202	0.55	70	54.72 (54.75)	3.89 (3.86)	24.54 (24.50)	11.24 (11.27)

Table 2: Antimicrobial activity of synthesized compounds

Comp.	Zone of inhibition at 100 µg/ml (in mm)			
	Antibacterial		Antifungal	
	E. coli	*S.* aureus	*A.* niger	*C.* albicans
R_1	13	15	**23**	**24**
R_2	16	18	16	13
R_3	**22**	**21**	**18**	**20**
R_4	13	10	12	16
R_5	17	12	15	13
R_6	15	14	14	16
R_7	**19**	**22**	**21**	**23**
R_8	**20**	**21**	16	15
R_9	**21**	**19**	**23**	**19**
R_{10}	16	14	13	14
Norfloxacin	**23**	**24**	**--**	**--**
Griseofulvin	**--**	**--**	**25**	**26**

Table 3: Analgesic activity of synthesized compounds

Comp.	Basal time in seconds (Mean±SEM)	Reaction time in seconds (Mean±SEM)			Percentage inhibition
		0. 5 h	1 h	1.5 h	
Control	2.867±0.092	2.867±0.094	2.868±0.090	2.868±0.089	00.00
Std	2.517±0.055	5.775±0.091***	8.162±0.084***	10.27±0.098***	62.10
R_1	2.612±0.099	4.857±0.086***	7.110±0.091***	7.733±0.095***	38.17
R_2	2.408±0.107	4.960±0.100***	6.287±0.090***	6.713±0.086***	34.46
R_3	2.623±0.098	5.087±0.092***	7.172±0.099***	7.518±0.093***	40.39
R_4	2.417±0.081	3.935±0.095***	7.348±0.104***	8.177±0.081***	44.70
R_5	2.532±0.094	4.643±0.059***	7.212±0.082***	6.520±0.039***	31.54
R_6	2.662±0.073	5.120±0.089***	6.687±0.095***	8.260±0.039***	46.43
R_7	2.458±0.061	4.833±0.084***	6.455±0.105***	7.620±0.127***	41.15
R_8	2.448±0.053	4.850±0.090***	6.878±0.085***	8.733±0.095***	50.29
R_9	2.392±0.080	4.627±0.093***	7.023±0.076***	9.132±0.091***	53.39
R_{10}	2.355±0.080	4.408±0.061***	5.718±0.096***	6.295±0.050***	29.66

Significance levels *P<0.05, **P<0.01, ***P<0.001 as compared with the respective control

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