A Simple and Efficient Procedure for Synthesis of Biologically Active 1,2,4-Triazolo-[3,4-b]-1,3,4-thiadiazole -2-aryl-thiazolidine-4-one Derivatives

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Abstract

Some new and biologically active [1,2,4] triazolo [3,4-b][1,3,4] thiadiazole-2-aryl-thiazolidinone-4-ones were synthesized by reaction of Schiff bases with mercapto acetic acid in presence of THF with adding anhydrous $ZnCl_2$. The structure of the synthesized compounds have been estabilished on the bases of IR, PMR, CMR and elemental analysis. The compounds have been evaluated for antibacterial activity against B. subtilis, S. aureus, P. aeruginosa and E. coli.

Key words: Synthesis, Biological activity,Triazolo, Thiadiazole, thiazolidinones

Introduction

In the last few decades, the chemistry of heterocycles bearing a triazole or 1,3,4-thiadiazole moiety are reported to show a wide spectrum of biological activity[1-8] such as antibacterial[9], anti aggregatory agent[10], antiviral[11] and anti-inflammatory[12] activities. In addition, the N-bridged heterocycles derived from 1,2,4-triazoles have applications in field of medicine, agriculture and industry.[13] 1,3,4-Thiadiazoles exhibit broad spectrum of biological activites possibly due to the presence of toxophoric N-C-S moiety[14]. They found applications as antibacterial, antitumor, anti inflammatory agents, pesticides, herbicides, dyes, lubricants and analytical reagents.[15] The [1,2,4]-triazolo-[3,4-b]-[1,3,4]-thiadiazole derivatives obtained by fusing the biolibale [1,2,4]-triazole and [1,3,4]-thiadiazole ring together, are reported to possess anti bacterial, antifungal, CNS depressant, anti viral, analgesic and plant growth regulatory effects.[16] In literature revealed, some derivatives of [1,2,4]-triazolo-[3,4-b]-[1,3,4]-thiadiazoles have been show as optically active with L-Amino acid.[17]

The 4-carbonyl derivative of thiazolidine is known as 4-thiazolidinone. 4-thiazolidinones have been well studied and a variety of biological activities have been reported for a large number of their derivatives. Suchasantibacterial[18], antimicrobial[19], anti fungal[20], anti anthelmintic[21], anti infiammatory[22], anti tubercular [23] and diuretic agents[24], anti thyroid [25] and as a local anaesthetic .

We report here in the reaction of Schiff bases with mercapto acetic acid in presence of THF with adding anhydrous $ZnCl_2$ to give biologically active [1,2,4] triazolo [3,4-b][1,3,4] thiadiazole-2-aryl-thiazolidinone-4-ones with the hope of to obtained compounds with good yield.

Material and Methods

Melting points were determined using an electro thermal digital apparatus and are uncorrected. Purity of the compound was checked by thin layer chromatography (TLC). IR spectra were prepared on a FT-IR spectrophotometer using KBr discs. ^1H PMR spectra were recorded on Bruker spectrophotometer (300 MHz) in DMSO-d_6 or $CDCl_3$ using TMS as an internal standard.

Synthesis of methyl benzoate (1): Benzoic acid (0.01 mole) in 20 ml of methanol and 0.5 ml conc. Sulfuric acid was refluxed for 12 hrs. and poured into ice. The product was isolated and treated with standard sodium bicarbonate solution to give desired compounds.

Synthesis of benzoic acid hydrazide (2): A mixture methyl benzoate (0.01 mole) and hydrazine hydrate (0.5 g, 0.01 mole) was heated for 9 hrs. and poured into ice. The product was isolated and crystallized from ethanol.

Synthesis of potassium-benzoic acid hydrazide dithiocarbamate (3): A mixture of benzoic acid hydrazide (0.01 mole), KOH (0.84 g, 0.015 mole) and 1.5 ml CS_2 in absolute alcohol was stirred for 21 hrs. and product was isolated from diethyl ether.

Synthesis of 4-amino-5-phenyl-4H-1,2,4-triazole-3-thiol (4): Potassium salt (0.01 mole) was taken in hydrazine hydrate and heated up to the evolution of H_2S gas cussed nearly 5 hrs. in oil bath. The reaction mixture was poured into crushed ice and treated with glacial Acetic acid .The product was filtered and purified by KOH treatment and crystallized from ethanol.

Synthesis of 3-(phenyl)-6-(4-N-acetylamino phenyl) [1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (5): A mixture of n-acetyl-p-amino benzoic acid (0.01 mole) and 4-amino-5-phenyl-4H-1,2,4-triazole-3-thiol (0.01 mole) in $POCl_3$ (25 ml) was refluxed for 10 hrs. The reaction mixture was poured into crushed ice and thus solid separated out was filtered, washed with water and crystallized from ethanol.

Synthesis of 3-(phenyl)-6-(4-amino phenyl) [1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (ATT) (6): 3-(phenyl)-6-(4-N-acetylamino phenyl) [1,2,4] triazolo[3,4-b][1,3,4]thiadiazole was hydrolysed by refluxing with 75 ml of ethanol containing 15 ml of concentrated HCl for 4-5 hrs. it was then poured into ice-cold water and finally made just alkaline with liquid ammonia. The resultant product 3-(phenyl)-6-(4-amino phenyl) [1,2,4] triazolo [3,4-b] [1,3,4] thiadiazole (ATT) is filtered off and washed with water and air dried. It was then recrystallised from ethanol.

Synthesis of Arylidine-[3,6-(diphenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole] (7a-h): A mixture of equimolar amount of 3-(phenyl)-6-(4-amino phenyl) [1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (0.01 mole) and various aromatic aldehydes (0.01mole) in 50 ml acetic acid and refluxed for about 10-12 hrs. on oil bath. The reaction mixture was cooled and it was poured in to ice water and extracted with ethyl acetate and water and finally dried over anhydrous sodium sulfate. The solvent was evaporated to give the solid product. It was crystallized from ethyl acetate hexane using decolorizing charcoal to give various anils

Synthesis of 3-[(3,6-diphenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole]-2-aryl-thiazolidine-4-ones (8a-h): A mixture of Schiff bases (7a-h) (0.01 mole) in THF (30 ml) and mercapto acetic acid (thioglycolic acid) (0.01 mole) with a pinch of anhydrous $ZnCl_2$ was refluxed for 12 hours. The solvent was then removed to get a residue, which was dissolved in benzene and passed through column of silica gel using benzene : chloroform (8:2;v/v) mixture as eluent. The eluate was concentrated and the product crystallized from alcohol to give 4-thiazolidinones(8a-h).

Spectral Analysis of Synthesized compounds (8a-h)

(1) 3-[(3,6-diphenyl)-[1,2,4]triazolo[3,4-b][1,3,4]-thiadiazole]-2-phenyl-thiazolidin-4-one (8a): I.R. (KBr, cm-1): 3030, 1500 (aromatic C-H), 1600, 1680 (C=O of thiazolidinone). PMR (δ ppm): 6.12-7.8 (m, aromatic), 3.1 (2H of CH_2 for thiazolidinone), 5.35 (H of C_2H for thiazolidinone). ^{13}CMR (δ ppm): 113-130 (benzene),136-145 (triazolo-thiadiazole), 169 (C=O), 36 (CH_2), 46 (CH).

(2) 3-[(3,6-diphenyl)-[1,2,4]triazolo[3,4-b][1,3,4]-thiadiazole]-2-(4-methoxyphenyl)-thiazolidin-4-one (8b): I.R. (KBr, cm-1): 3030, 1500 (aromatic C-H), 1600, 1690 (pyridine ring, C=O of thiazolidinone), 1200 (Ar-O-CH_3). PMR (δ ppm): 6.12-7.8 (m, aromatic), 3.2 (2H of CH_2 for thiazolidinone), 5.35 (H of C_2H for thiazolidinone), 3.35 (3H of CH_3 of thiazolidinone). ^{13}CMR (δ ppm): 113-131 (benzene),135-145 (triazolo-thiadiazole), 169 (C=O), 36 (CH_2), 46 (CH), 56 (CH_3).

(3) 3-[(3,6-diphenyl)-[1,2,4]triazolo[3,4-b][1,3,4]-thiadiazole]-2-(4-hydroxyphenyl)-thiazolidin-4-one (8c): I.R. (KBr, cm-1): 3030,

1500 (aromatic C-H), 1600, 1690 (C=O of thiazolidinone), 3200-2600 (broad, OH stretching), 2880, 2920 (CH_2 stretching). PMR (δ ppm): 6.12-7.8 (m, aromatic), 3.2 (2H of CH_2 for thiazolidinone), 5.35 (H of C_2H for thiazolidinone), 3.9 (H of OH). ^{13}CMR (δ ppm): 113-132 (benzene),135-145 (triazolo-thiadiazole), 169 (C=O), 36 (CH_2), 46 (CH).

(4) 3-[(3,6-diphenyl)-[1,2,4]triazolo[3,4-b][1,3,4]-thiadiazole]-2-(2-hydroxyphenyl)-thiazolidin-4-one (8d): I.R. (KBr, cm-1): 3030, 1500 (aromatic C-H), 1600, 1690 (C=O of thiazolidinone), 3200-2600 (broad, OH stretching), 2880, 2920 (CH_2 stretching). PMR (δ ppm): 6.12-7.8 (m, aromatic), 3.1 (2H of CH_2 for thiazolidinone), 5.35 (H of C_2H for thiazolidinone), 3.9 (H of OH). ^{13}CMR (δ ppm): 113-133 (benzene),135-147 (triazolo-thiadiazole), 169 (C=O), 36 (CH_2), 46 (CH).

(5) 3-[(3,6-diphenyl)-[1,2,4]triazolo[3,4-b][1,3,4]-thiadiazole]-2-(2-methylphenyl)-thiazolidin-4-one (8e): I.R. (KBr, cm-1): 3030, 1500 (aromatic C-H), 1600, 1690 (C=O of thiazolidinone), 2950,1370 (CH_3 stretching), 2880, 2920 (CH_2 stretching). PMR (δ ppm): 6.2-7.9 (m, aromatic), 3.2 (2H of CH_2 for thiazolidinone), 2.1 (3H of CH_3), 5.35 (H of C_2H for thiazolidinone) . ^{13}CMR (δ ppm): 113-134 (benzene),135-148 (triazolo-thiadiazole), 169 (C=O), 36 (CH_2), 46 (CH), 25 (CH_3).

(6) 3-[(3,6-diphenyl)-[1,2,4]triazolo[3,4-b][1,3,4]-thiadiazole]-2-(methylenedioxyphenyl)-thiazolidin-4-one (8f): I.R. (KBr, cm-1): 3030, 1500 (aromatic C-H), 1600 (thiazole ring), 1690 (C=O of thiazolidinone), 1200 (Aryl-alkyl ether), 2880, 2920 (CH_2 stretching). PMR (δ ppm): 6.15-7.8 (m, aromatic), 5.35 (H of C_2H for thiazolidinone), 5.35 (2H for CH_2 of -O-CH_2-O-), 3.2 (2H for C_5H). ^{13}CMR (δ ppm): 114-130(benzene),135-149 (triazolo-thiadiazole), 169 (C=O), 36 (CH_2), 46 (CH), 95 (O-CH_2-O).

(7) 3-[(3,6-diphenyl)-[1,2,4]triazolo[3,4-b][1,3,4]-thiadiazole]-2-(3-methylenedioxyphenyl)-thiazolidin-4-one (8g): I.R. (KBr, cm-1): 3030, 1500 (aromatic C-H), 1600,1680 (C=O of thiazolidinone), 3200-2600 (OH), 1200 (Aryl-alkyl ether), 2880, 2920 (CH_2 stretching). PMR (δ ppm): 6.12-7.9 (m, aromatic), 3.2 (2H for C_5H), 5.35 (H of C_2H for thiazolidinone), 3.35 (3H for –OCH_3), 3.9 (H for OH). ^{13}CMR (δ ppm): 113-135 (benzene),135-150 (triazolothiadiazole), 169 (C=O), 36 (CH_2), 46 (CH), 56 (CH_3).

(8) 3-[(3,6-diphenyl)-[1,2,4]triazolo[3,4-b][1,3,4]-thiadiazole]-2-(3,4-diethoxyphenyl)-thiazolidin-4-one (8h): I.R. (KBr, cm-1): 3030, 1500 (aromatic C-H), 1600,1680 (C=O of thiazolidinone), 1200 (Aryl-alkyl ether), 2880, 2920 (CH_2 stretching). PMR (δ ppm): 7.2-8.1 (m, aromatic), 3.25 (2H for C_5H), 3.35 (H for C_2H), 2.1-2.5 (6H for $2CH_3$), 2.89-3.18 (4H for 2 CH_2). ^{13}CMR (δ ppm): 113-136 (benzene),135-151 (triazolo thiadiazole), 169 (C=O), 36 (CH_2), 14 (CH_3), 58 (OCH_2).

Results and Discussion

All the new derivatives of 1,2,4-triazolo[3,4-b][1,3,4] thiadiazoles-2-aryl-thiazolidinone-4-ones were synthesized and the structures were established by means of IR, ^{1}H NMR spectral data as well as elemental analysis.

All the thiazolidinone-4-ones were evaluated for anti-microbial activity. The newley synthesized compounds (8a-h) were obtained by the treatment of Schiff bases in THF and mercapto acetic acid (thio-glycolic acid) with a pinch of anhydrous $ZnCl_2$ was refluxed for 12 hours. All the synthesized structures showed satisfactory result. The IR data of the compounds clearly showed a strong C=O stretching band around 1600 cm^{-1} and aromatic C-H in thiazolidinone of absorption band around 3030, 1500 cm^{-1}. The PMR also confirms the presence of shift value at 3.1 for CH_2 in thiazolidinone and 5.35 for C_2H also in thiazolidinone groups respectively. Antibacterial screening of newly synthesized compounds was carried out against *B. subtilis, S. aureus, P. aeruginosa and E. coli* in DMF solvent using cup-plate method. From the results obtained in the biological activity, it was observed that, the compound 8b, 8c, 8d and 8h were shown significant

activities and compound 8a, 8e, 8f and 8g have shown moderate activity.

Biological evaluation: Cup-plate agar diffusion method[12,13] was employed for *in vitro* study of antibacterial. Efficacy of the target compounds against *B. subtilis*, *S. aureus, Ps. aeruginosa and E. coli* in DMF solvent.

The study has been conducted according to the method adopted by Nutrient agar broth was melted in a water bath and cooked to 45^{O}C with gentle shaking to bring about uniform cooling. It was inoculated with 0.5-0.6 ml of 24 hour old culture especially and mixed well by gentle shaking before pouring on the sterilized Petri dish (25 ml each). The poured material was allowed to set (1.5 hour) and there after the "cups" were made by punching into the agar surface with a sterile cork borer and sooping out the punched part of agar. Into this "cups" 0.1 ml of test solution (prepared by dissolving 10gm of sample in 10ml DMF) was added by sterile micropipette. The plates were noted. Ampicillin, Tetracycline, Gentamycin, and Chloramphenicol were used as standard drugs. The biological activity test data are presented in Table 2. The compound 8b, 8c, 8d and 8h were shown significant activities and compound 8a, 8e, 8g and 8h have shown moderate activity.

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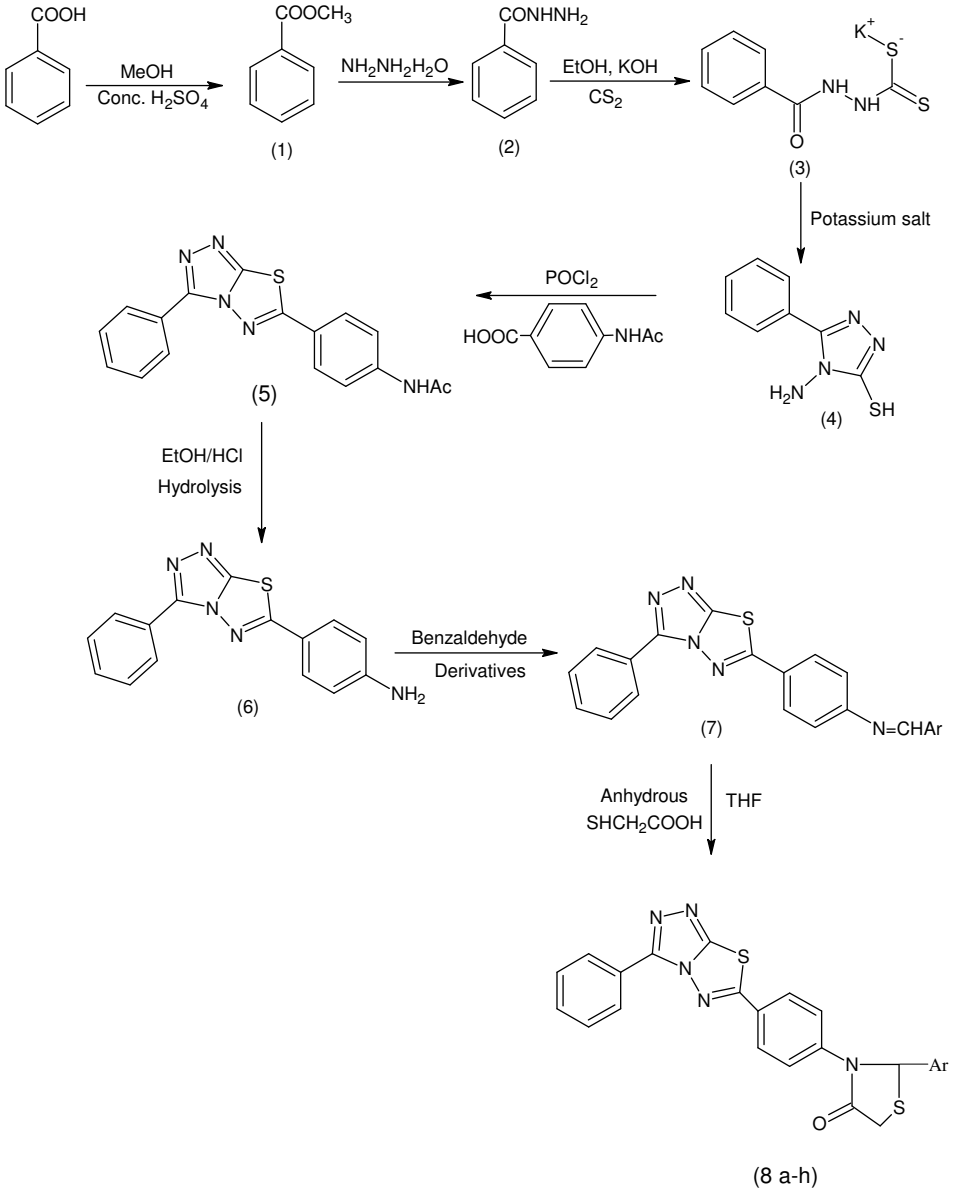


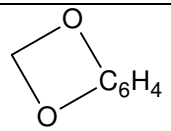
Sr. No	R	Molecular Formula	Molecular Weight	M.P. °C	Yield %	% of C, H, N, S Cal / Found			
						C	H	N	S
8a	C_6H_6	$C_{24}H_{17}N_5OS_2$	455	152	63	63.2	3.7	15.3	14.3
						63.0	3.5	15.2	14.0
8b	4-OCH_3-C_6H_5	$C_{25}H_{19}N_5O_2S_2$	485	168	62	61.8	3.9	14.4	13.1
						61.5	3.8	14.2	13.0
8c	4-OH-C_6H_5	$C_{24}H_{17}N_5O_2S_2$	471	175	58	61.1	3.6	14.8	13.5
						61.0	3.5	14.6	13.2
8d	2-OH-C_6H_5	$C_{24}H_{17}N_5O_2S_2$	471	158	70	61.1	3.6	14.8	13.5
						61.0	3.5	14.5	13.2
8e	4-CH_3-C_6H_5	$C_{25}H_{19}N_5OS_2$	469	175	57	63.9	4.0	14.9	13.6
						63.7	4.2	14.7	13.5
8f		$C_{25}H_{17}N_5O_3S_2$	499	180	48	60.1	3.4	14.0	12.8
						60.0	3.2	13.9	12.6
8g	4-OH-3-OCH_3-C_6H_4	$C_{25}H_{19}N_5O_3S_2$	501	162	52	59.8	3.8	13.9	12.7
						59.5	3.7	13.6	12.2
8h	3-OC_2H_5-4-OC_2H_5-C_6H_4	$C_{28}H_{25}N_5O_3S_2$	543	161	53	61.8	4.6	12.8	11.7
						61.6	4.4	12.5	11.6

Table-1: Physical Constant of 3-[(3,6-diphenyl)-(1,2,4triazolo(3,4-b)(1,2,3)-thiadiazole]-2-aryl-thiazolidin-4-one (8a-h)

Table – 2: Antibactarial activity of standards and solvent (DMF)

Compounds	Zone of Inhibition in (mm)			
	Gram Positive		Gram Negative	
	B. Subtilis	***S. Aureus***	***E. Coli***	***Ps. Aeruginosa***
DMF	06	05	05	05
Ampicillin	19	15	20	21
Tetracycline	21	20	15	18
Gentamycine	20	18	19	22
Chloramphenicol	20	23	18	24

Tabel- 3: Antibacterial activity of 3-[(3,6-diphenyl)-[1,2,4]triazolo[3,4-b][1,3,4]-thiadiazole]-2-aryl-thiazolidin-4-one (8a-h)

Compounds	Zone of Inhibition in (mm)			
	Gram Positive		Gram Negative	
	B. Subtilis	***S. Aureus***	***E. Coli***	***Ps. Aeruginosa***
8a	09	09	16	08
8b	12	10	14	11
8c	10	13	11	15
8d	15	18	19	14
8e	07	09	10	08
8f	11	12	09	12
8g	08	09	10	12
8h	08	14	11	13