Scientific paper

Synthesis and Antimicrobial Activity of Some New 1,3,4-Thiadiazoles and 1,3,4-Thiadiazines Containing 1,2,4-Triazolo Nucleus

Dushyant H. Purohit, Bhavesh L. Dodiya, Renish M. Ghetiya, Piyush B. Vekariya and Hitendra S. Joshi*

Chemical Research Laboratory, Department of Chemistry, Saurashtra University, Rajkot 360005, India

** Corresponding author: E-mail: drhsjoshi49@gmail.com*

Received: 01-07-2010

Abstract

The desired fused ring system 3-(3-chlorophenyl)-6-aryl-5,6-dihydro[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles **5a**–**j** were synthesized by the reaction of 4-amino-5-(3-chlorophenyl)-4*H*-1,2,4-triazole-3-thiol and different aryl aldehydes in the presence of catalytic amount of *p*-TsOH in dry DMF, while 3-(3-chlorophenyl)-6-aryl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines **7a**–**j** were synthesized by using 4-amino-5-(3-chlorophenyl)-4*H*-1,2,4-triazole-3-thiol and different phenacyl bromides in dry methanol. Their IR, ^{1}H NMR, mass spectral data and elemental analyses were in accord with assigned structures. All the newly synthesized compounds were screened for their antimicrobial activity. Some of the compounds exhibited significant inhibition on bacterial and fungal growth as compared to standard drugs.

Keywords: 1,2,4-triazole, 1,3,4-thiadiazoles, 1,3,4-thiadiazines, *p*-TsOH, antimicrobial activity

1. Introduction

The therapeutic effects of compounds containing 1,3,4-thiadiazole and 1,3,4-thiadiazine rings have been well studied for a number of pathological conditions including inflammation,[1,2] pain[3] and hypertension.[4] Moreover, syntheses of 1,3,4-thiadiazoles and 1,3,4-thiadiazines have attracted widespread attention due to their diverse applications as antibacterial,[5,6] antimycobacterial,[7,8] antimycotic,[9,10] antifungal[11,12] and antidepressant agents.[13]

Compounds bearing the 1,2,4-triazole ring are well known as powerful antimicrobial,[14] anticonvulsant,[15] antidepressant,[16] antihypertensive,[17] antitumorial[18] and analgesic[19] agents.

Moreover, synthesis of triazole fused to 1,3,4-thiadiazole and 1,3,4-thiadiazine rings has attracted widespread attention due to their diverse applications as antimicrobial,[20,21] antidepressant, antiviral, antitumorial and anti-inflammatory agents, pesticides, herbicides, dyes, lubricant and analytical reagents.[22]

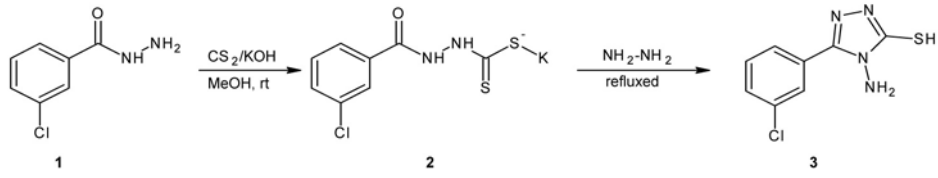
In view of the biological importance of these heterocycles a new series of 3-(3-chlorophenyl)-6-aryl-5,6-dihydro[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles **5a**–**j** and 3-(3-chlorophenyl)-6-aryl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines **7a**–**j** were synthesized and evaluated for their *in vitro* antimicrobial activity.

2. Results and Discussion

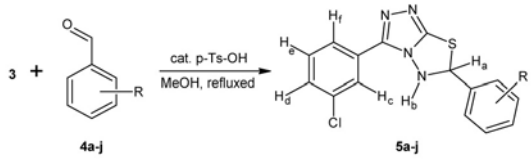
The starting 4-amino-5-(3-chlorophenyl)-4*H*-1,2,4-triazole-3-thiol (**3**) was obtained in two steps. First, 3-chlorobenzohydrazide (**1**) reacted with carbon disulphide in the presence of KOH to form potassium 3-chlorobenzyl dithiocarbamate (**2**) in a good yield (Scheme 1). A cyclization reaction between hydrazine hydrate and **2** gave 4-amino-5-(3-chlorophenyl)-4*H*-[1,2,4]-triazole-3-thiol (**3**).

The synthetic pathway followed in the preparation of 1,3,4-thiadiazoles **5a**–**j** and 1,3,4-thiadiazines **7a**–**j** is outlined in Schemes 2 and 3.

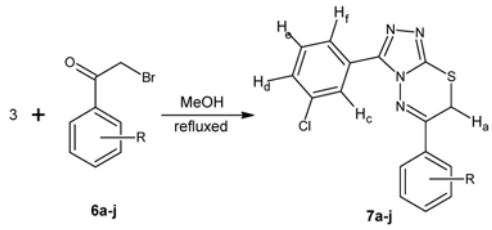
The required intermediates **1**[23] and **6a**–**j**[24] have been prepared by the literature methods. The compounds **5a**–**j** were synthesized by reacting 4-amino-5-(3-chlorophenyl)-4*H*-1,2,4-triazole-3-thiol (**3**) with different aryl aldehydes in the presence of a catalytic amount of *p*-TsOH in dry DMF. Further, compound **3** on reaction with different



Scheme 1: Synthesis of 1,2,4-triazole.



Scheme 2: Synthesis of 1,3,4-thiadiazoles **5a–j**.



Scheme 3: Synthesis of 1,3,4-thiadiazines **7a–j**.

phenacyl bromides in dry methanol gave compounds **7a–j**. The purity of all the new compounds was checked by TLC. The structures of the synthesized compounds were assigned on the basis of spectral data (IR, ^{1}H NMR, mass spectral analysis) and elemental analysis.

2. 1. Antimicrobial Activity

The antimicrobial activity was evaluated by using the cup-plate agar diffusion method[25] by measuring the zone of inhibition in mm. The antimicrobial activity was compared with standard drugs amoxicillin, ciprofloxacin, erythromycin, benzyl penicillin and antifungal activity was compared with greseofulvin.

2. 1. 1. Antibacterial Activity

The purified compounds were screened for their antibacterial activity using cup plate agar diffusion method. The nutrient agar broth prepared by the usual method was dispensed in 50 mL quantities of different conical flasks. Then, the 0.5 mL culture of each bacteria (*Bacillus megaterium* ATCC 14518, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853) in nutrient agar broth was added and inoculated at 37 °C for 24 h. The nutrient agar was melted at 100 °C and after cooling to 56 °C, was poured into petri plates of 13 cm diameter in quantities of 20 mL, and left on a flat surface to solidify and the surface of the medium was dried at 37 °C. Then, above subcultures of each bacteria were pipetted in to the nutrient agar plate. The cups (10 mm diameter) were formed by the help of borer in agar medium and filled with 0.04 mL (40 µg) solution of sample in DMF. The plates were incubated at 37 °C for 24 h and the control was also maintained with 0.04 mL of DMF in a similar manner. After the completion of the incubation period, the zone of inhibition of growth in the form of diameter in mm was measured (Table 1).

2. 1. 2. Antifungal Activity

Aspergillus niger ATCC 9029 was employed for testing antifungal activity using cup-plate agar diffusion method. The culture was maintained on sabourauds agar slants, sterilized sabourauds agar medium was inoculated with 72 h old 0.5 mL suspension of fungal spores in a separate flask.

The sabourauds agar was melted at 100 °C and after cooling to 56 °C, was poured into petri plates of 13 cm diameter in quantities of 20 mL, and left on a flat surface to solidify and the surface of the medium was dried at 37 °C. Then, above subculture of fungi was pipetted in to the sabourauds agar plate. The cups (10 mm diameter) were formed by the help of borer in agar medium and filled with 0.04 mL (40 µg) solution of sample in DMF. The plates were incubated at 30 °C for 48 h and the control was also maintained with 0.04 mL of DMF in a similar manner. After the completion of the incubation period, the zone of inhibition of growth in the form of diameter in mm was measured (Table 1).

Evaluation of the newly synthesized compounds **5c**, **5g–i**, **7c**, **7f**, **7g** and **7i** were active against *B. megaterium*. Similarly, compounds **5a**, **5g**, **5h**, **5i**, **7c**, **7f**, **7g** and **7i** were most potent against *P. aeruginosa*. Also compounds **5g–i**, **7f**, **7g** and **7i** were highly potent against *E. coli*. Compounds **5g**, **5i**, **7g** and **7i** were most active against *S. aureus*. Against *A. niger* compounds **5c**, **5g**, **5i**, **7g** and **7i** were active. Remaining compounds did not show any promising activity against tested bacteria and fungi.

3. Experimental Section

Melting points were determined on electro thermal apparatus using open capillaries and are uncorrected. Thin layer chromatography was accomplished on 0.2 mm pre-

Table 1. Antimicrobial screening results of compounds **5a–j** and **7a–j**

Compound	R	Zones of inhibition in mm				
		Antibacterial activity				Antifungal activity
		B. megaterium	*S. aureus*	*E. coli*	*P. aeruginosa*	*A. niger*
5a	C_6H_5-	16	18	10	14	15
5b	4-OCH_3-C_6H_4-	15	14	15	12	11
5c	2,4-$(CH_3)_2$-C_6H_3-	19	17	16	12	20
5d	4-CH_3-C_6H_4-	15	16	08	10	16
5e	2-OH-C_6H_4-	12	13	15	15	12
5f	4-OH-C_6H_4-	13	14	13	16	10
5g	3-NO_2-C_6H_4-	20	19	18	18	19
5h	2-NO_2-C_6H_4-	21	20	18	15	16
5i	4-F-C_6H_4-	24	22	20	20	18
5j	3-Cl-C_6H_4-	12	11	16	10	13
7a[26]	C_6H_5-	15	16	14	13	14
7b[27]	4-OCH_3-C_6H_4-	13	12	10	13	16
7c	2-SCH_3-C_6H_4-	19	19	16	15	16
7d	4-CH_3-C_6H_4-	11	15	11	10	11
7e	2-OH-C_6H_4-	10	11	11	12	15
7f[26]	4-NO_2-C_6H_4-	20	19	18	16	13
7g	4-F-C_6H_4-	23	22	20	19	18
7h[26]	4-Cl-C_6H_4-	16	12	15	15	10
7i	4-SO_2CH_3-C_6H_4-	22	24	19	18	21
7j	3-Cl-C_6H_4-	15	14	15	14	14
Amoxicillin	–	20	21	18	20	00
Benzyl penicillin	–	21	24	19	18	00
Ciprofloxacin	–	22	25	24	18	00
Erythromycin	–	25	25	25	17	00
Greseofulvin	–	00	00	00	00	24

coated plates of silica gel G60 F_{254} (Merck, St. Louis, MO, USA). Visualization was made with UV light (254 and 365nm) or with iodine vapor. IR spectra were recorded on a FTIR-8400 spectrophotometer (Shimadzu, Kyoto, Japan), using DRS probe, all data are given for stretching frequencies. ^{1}H NMR spectra were recorded on a Bruker AVANCE II (400 MHz) spectrometer (Bruker, Rheinstetten, Germany), in $CDCl_3$. Chemical shifts are expressed in δ ppm downfield from TMS as an internal standard. Mass spectra were determined using direct inlet probe on a GCMS-QP 2010 mass spectrometer (Shimadzu, Kyoto, Japan). Elemental analysis was performed on a Carlo-Erba EA 1108 elemental analyzer (Waltham, MA, USA).

Synthesis of Potassium 3-Chlorobenzyl Dithiocarbamate (2).

To a mixture of potassium hydroxide (8.40 g, 0.15 mol) and 3-chlorobenzohydrazide (17.0 g, 0.1 mol) in methanol (25 mL), carbon disulphide (11.4 g, 0.15 mol) was added. This mixture was stirred for 12–14 h. It was then diluted with dry ether (200 mL) and the solid thus obtained was filtered and washed with ether and dried to give potassium 3-chlorobenzyl dithiocarbamate (**2**). There is no need to purify the salt for further reaction.

Synthesis of 4-Amino-5-(3-chlorophenyl)-4*H*-1,2,4-triazole-5-thiol (3).

A suspension of the potassium salt **2** (24.5 g, 0.1 mol), hydrazine hydrate (10 mL, 0.2 mol) and water (2 mL) was refluxed with stirring for 3 h. The color of the reaction mixture changed to green, hydrogen sulfide was evolved (lead acetate paper and odor) and a homogeneous solution resulted. The solution was diluted with cold water (100 mL) and neutralized with glacial acetic acid; a white solid precipitated. The product was filtered, washed with cold water and crystallized from dioxane, yield 60%, m.p. 190 °C.

Synthesis of 3-(3-Chlorophenyl)-6-aryl-5,6-dihydro [1,2,4]triazole[3,4-*b*][1,3,4]thiadiazoles 5a–j.

A mixture of 4-amino-5-(3-chlorophenyl)-4*H*-1,2,4-triazole-5-thiol (**3**) (2.26 g, 0.01 mol) and different aryl aldehydes **4a–j** (0.01 mol) was dissolved in dry DMF. Then catalytic amount of *p*-TsOH was added. The resulting mixture was refluxed with continuous stirring. After complete reaction (8–10 h, monitoring by TLC) it was cooled to the room temperature, then ice-cold water was added and extracted into ethyl acetate. The solvent was removed in vacuum and the resulting crude product was crystallized from ethanol to give the pure compounds **5a–j**.

3-(3-Chlorophenyl)-6-phenyl-5,6-dihydro[1,2,4] triazole[3,4-*b*][1,3,4]thiadiazole (5a).

Yield 61%; mp 180–182 °C; IR (KBr) *ν* 3463 (N–H), 3087 (C–H), 1591 (C=N), 1509 (C=C), 1168 (C–N), 1071 (N–N), 781 (C–Cl), 693 (C–S) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.01 (s, 1H, Ar-Hc), 7.57–7.77 (m, 3H, Ar-Hd,e,f), 7.21–7.43 (m, 5H, Ar-H), 6.25 (s, 1H, Ha), 2.95 (s, 1H, NH); MS *m/z* 315 [M^+]; Anal. Calcd for $C_{15}H_{11}ClN_4S$: C, 57.23; H, 3.52; N, 17.80. Found: C, 57.11; H, 3.43; N, 17.78.

3-(3-Chlorophenyl)-6-(4-methoxyphenyl)-5,6-dihydro [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (5b). Yield 60%; mp 264–266 °C; IR (KBr) *ν* 3440 (N–H), 3070 (C–H), 1601 (C=N), 1535 (C=C), 1252 (C–O–C), 1166 (C–N), 1027 (N–N), 774 (C–Cl), 700 (C–S) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.09 (s, 1H, Ar-Hc), 7.62–7.83 (m, 3H, Ar-Hd,e,f), 7.36–7.49 (m, 4H, Ar-H), 6.17 (s, 1H, Ha), 3.64 (s, 3H, OCH_3), 2.91 (s, 1H, N-H); MS *m/z* 345 [M^+]; Anal. Calcd for $C_{16}H_{13}ClN_4OS$: C, 55.73; H, 3.80; N, 16.25. Found: C, 55.59; H, 3.75; N, 16.17.

3-(3-Chlorophenyl)-6-(2,4-dimethoxyphenyl)-5,6-dihydro[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (5c). Yield 68%; mp 220–222 °C; IR (KBr) *ν* 3482 (N–H), 3029 (C–H), 1617 (C=N), 1541 (C=C), 1243 (C–O–C), 1124 (C–N), 1058 (N–N), 785 (C–Cl), 707 (C–S) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.13 (s, 1H, Ar-Hc), 7.59–7.74 (m, 3H, Ar-Hd,e,f), 7.33–7.59 (m, 3H, Ar-H), 6.23 (s, 1H, Ha), 3.71 (s, 6H, OCH_3), 2.85 (s, 1H, N-H); MS *m/z* 375 [M^+]; Anal. Calcd for $C_{17}H_{15}ClN_4O_2S$: C, 54.47; H, 4.03; N, 14.95. Found: C, 54.36; H, 3.96; N, 14.83.

3-(3-Chlorophenyl)-6-(4-methylphenyl)-5,6-dihydro [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (5d). Yield 67%; mp 180–181 °C; IR (KBr) *ν* 3433 (N–H), 3107 (C–H), 1623 (C=N), 1540 (C=C), 1092 (C–N), 993 (N–N), 791 (C–Cl), 703 (C–S) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.14 (s, 1H, Ar-Hc), 7.45–7.71 (m, 3H, Ar-Hd,e,f), 7.16–7.36 (m, 4H, Ar-H), 6.31 (s, 1H, Ha), 2.74 (s, 1H, NH), 2.35 (s, 3H, CH_3); MS *m/z* 329 [M^+]; Anal. Calcd for $C_{16}H_{13}ClN_4S$: C, 58.44; H, 3.98; N, 17.04. Found: C, 58.39; H, 3.83; N, 16.96.

2-[3-(3-Chlorophenyl)-5,6-dihydro[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-6-yl]phenol (5e). Yield 63%; mp 198–200 °C; IR (KBr) *ν* 3568 (O–H), 3478 (N–H), 3033 (C–H), 1593 (C=N), 1460 (C=C), 1134 (C–N), 1030 (N–N), 774 (C–Cl), 687 (C–S) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.11 (s, 1H, Ar-Hc), 7.64–7.83 (m, 3H, Ar-Hd,e,f), 7.30–7.52 (m, 4H, Ar-H), 6.13 (s, 1H, Ha), 4.71 (s, 1H, OH), 2.97 (s, 1H, NH); MS *m/z* 331 [M^+]; Anal. Calcd for $C_{15}H_{11}ClN_4OS$: C, 54.46; H, 3.35; N, 16.94. Found: C, 54.33; H, 3.28; N, 16.84.

4-[3-(3-Chlorophenyl)-5,6-dihydro[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-6-yl]phenol (5f). Yield 68%; mp 210–214 °C; IR (KBr) *ν* 3593 (O–H), 3511 (N–H), 3078 (C–H), 1583 (C=N), 1478 (C=C), 1147 (C–N), 1037 (N–N), 779 (C–Cl), 684 (C–S) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.16 (s, 1H, Ar-Hc), 7.68–7.91 (m, 3H, Ar-Hd,e,f), 7.26–7.47 (m, 4H, Ar-H), 6.18 (s, 1H, Ha), 4.43 (s, 1H, OH), 2.93 (s, 1H, NH); MS *m/z* 331 [M^+]; Anal. Calcd for $C_{15}H_{11}ClN_4OS$: C, 54.46; H, 3.35; N, 16.94. Found: C, 54.37; H, 3.30; N, 16.86.

3-(3-Chlorophenyl)-6-(3-nitrophenyl)-5,6-dihydro [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (5g). Yield 69%; mp 230–231 °C; IR (KBr) *ν* 3451 (N–H), 3063 (C–H), 1610 (C=N), 1521 (C=C), 1087 (C–N), 1018 (N–N), 781 (C–Cl), 713 (C–S) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.97 (s, 1H, Ar-Hc), 7.63–7.88 (m, 3H, Ar-Hd,e,f), 7.33–7.58 (m, 4H, Ar-H), 6.37 (s, 1H, Ha), 3.04 (s, 1H, NH); MS *m/z* 360 [M^+]; Anal. Calcd for $C_{15}H_{10}ClN_5O_2S$: C, 50.07; H, 2.80; N, 19.46. Found: C, 50.00; H, 2.67; N, 19.39.

3-(3-Chlorophenyl)-6-(2-nitrophenyl)-5,6-dihydro [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (5h). Yield 71%; mp 193–195 °C; IR (KBr) *ν* 3468 (N–H), 3091 (C–H), 1588 (C=N), 1498 (C=C), 1107 (C–N), 1031 (N–N), 797 (C–Cl), 709 (C–S) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.06 (s, 1H, Ar-Hc), 7.49–7.80 (m, 3H, Ar-Hd,e,f), 7.29–7.40 (m, 4H, Ar-H), 6.33 (s, 1H, Ha), 3.11 (s, 1H, NH); MS *m/z* 360 [M^+]; Anal. Calcd for $C_{15}H_{10}ClN_5O_2S$: C, 50.07; H, 2.80; N, 19.46. Found: C, 49.96; H, 2.71; N, 19.35.

3-(3-Chlorophenyl)-6-(4-fluorophenyl)-5,6-dihydro [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (5i). Yield 60%; mp 189–191 °C; IR (KBr) *ν* 3379 (N–H), 3015 (C–H), 1580 (C=N), 1483 (C=C), 1129 (C–N), 1047 (N–N), 785 (C–Cl), 681 (C–S) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.04 (s, 1H, Ar-Hc), 7.45–7.73 (m, 3H, Ar-Hd,e,f), 7.13–7.32 (m, 4H, Ar-H), 6.18 (s, 1H, Ha), 3.07 (s, 1H, NH); MS *m/z* 333 [M^+]; Anal. Calcd for $C_{15}H_{10}ClFN_4S$: C, 54.14; H, 3.03; N, 16.84. Found: C, 54.03; H, 2.91; N, 16.77.

3,6-Bis(3-chlorophenyl)-5,6-dihydro[1,2,4]triazolo [3,4-*b*][1,3,4]thiadiazole (5j). Yield 73%; mp 195–197 °C; IR (KBr) *ν* 3417 (N–H), 3029 (C–H), 1561 (C=N), 1510 (C=C), 1124 (C–N), 1053 (N–N), 796 (C–Cl), 694 (C–S) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.95 (s, 1H, Ar-Hc), 7.52–7.84 (m, 3H, Ar-Hd,e,f), 7.28–7.40 (m, 4H, Ar-H), 6.27 (s, 1H, Ha), 2.78 (s, 1H, NH); MS *m/z* 350 [M^+]; Anal. Calcd for $C_{15}H_{10}Cl_2N_4S$: C, 51.59; H, 2.89; N, 16.04. Found: C, 51.53; H, 2.75; N, 15.89.

Synthesis of 3-(3-Chlorophenyl)-6-aryl-7*H*-[1,2,4] triazolo[3,4-*b*][1,3,4]thiadiazines 7a–j.

The mixture of 4-amino-5-(3-chlorophenyl)-4*H*-1,2,4-triazole-5-thiol (**3**) (2.26 g, 0.01 mol) and different phenacyl bromides **6a–j** (0.01 mol) were dissolved in dry

methanol (50 mL). The mixture was heated under reflux. After the completion of the reaction (4–5 h, monitoring by TLC) the content was cooled to the room temperature, the reaction mixture was neutralized with aqueous potassium carbonate solution. The solution was extracted with dichloromethane, and the organic layer was dried over anhydrous Na_2SO_4. After evaporating the solvent, the crude product was purified by silica gel column using hexane–ethyl acetate (2:2) as eluent to offer pure products **7a**–**j**.

3-(3-Chlorophenyl)-6-phenyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine (7a)[26]**.** Yield 69%; mp 178–180 °C; IR (KBr) *ν* 3079 (C–H), 1683 (C=N), 1542 (C=C), 1139 (C–N), 1043 (N–N), 785 (C–Cl), 700 (C–S) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.14 (s, 1H, Ar-Hc), 7.63–7.87 (m, 3H, Ar-Hd,e,f), 7.11–7.37 (m, 5H, Ar-H), 4.22 (s, 2H, Ha); MS *m/z* 327 [M^+]; Anal. Calcd for $C_{16}H_{11}ClN_4S$: C, 58.80; H, 3.39; N, 17.14. Found: C, 58.61; H, 3.29; N, 17.02.

3-(3-Chlorophenyl)-6-(4-methoxyphenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine (7b)[27]. Yield 60%; mp 210–212 °C; IR (KBr) *ν* 3029 (C–H), 1691 (C=N), 1460 (C=C), 1257 (C–O–C), 1170 (C–N), 1058 (N–N), 781 (C–Cl), 687 (C–S) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.09 (s, 1H, Ar-Hc), 7.57–7.81 (m, 3H, Ar-Hd,e,f), 7.27–7.43 (m, 4H, Ar-H), 4.32 (s, 2H, Ha), 3.91 (s, 3H, OCH_3); MS *m/z* 357 [M^+]; Anal. Calcd for $C_{17}H_{13}ClN_4OS$: C, 57.22; H, 3.67; N, 15.70. Found: C, 57.08; H, 3.59; N, 15.56.

3-(3-Chlorophenyl)-6-[4-(methylthio)phenyl]-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine (7c). Yield 66%; mp 168–171 °C; IR (KBr) *ν* 3018 (C–H), 1627 (C=N), 1478 (C=C), 1153 (C–N), 1027 (N–N), 796 (C–Cl), 691 (C–S) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.11 (s, 1H, Ar-Hc), 7.70–7.93 (m, 3H, Ar-Hd,e,f), 7.19–7.35 (m, 4H, Ar-H), 4.37 (s, 2H, Ha), 3.15 (s, 3H, SCH_3); MS *m/z* 373 [M^+]; Anal. Calcd for $C_{17}H_{13}ClN_4S_2$: C, 54.76; H, 3.51; N, 15.02. Found: C, 54.36; H, 3.41; N, 14.85.

3-(3-Chlorophenyl)-6-(4-methylphenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine (7d). Yield 70%; mp 206–207 °C; IR (KBr) *ν* 3062 (C–H), 1593 (C=N), 1511 (C=C), 1134 (C–N), 1071 (N–N), 776 (C–Cl), 703 (C–S) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.17 (s, 1H, Ar-Hc), 7.61–7.97 (m, 3H, Ar-Hd,e,f), 7.14–7.29 (m, 4H, Ar-H), 4.15 (s, 2H, Ha), 2.58 (s, 3H, CH_3); MS *m/z* 341 [M^+]; Anal. Calcd for $C_{17}H_{13}ClN_4S$: C, 59.91; H, 3.84; N, 16.44. Found: C, 59.73; H, 3.64; N, 16.38.

2-[3-(3-Chlorophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine-6-yl]phenol (7e). Yield 67%; mp 210–213 °C; IR (KBr) *ν* 3487 (O–H), 3073 (C–H), 1643 (C=N), 1483 (C=C), 1107 (C–N), 1060 (N–N), 794 (C–Cl), 680 (C–S) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.10 (s, 1H, Ar-Hc), 7.74–7.85 (m, 3H, Ar-Hd,e,f), 7.23–7.39 (m, 4H, Ar-H), 4.78 (s, 2H, Ha), 4.29 (s, 1H, OH); MS *m/z* 343 [M^+]; Anal. Calcd for $C_{16}H_{11}ClN_4OS$: C, 56.06; H, 3.23; N, 16.34. Found: C, 55.97; H, 3.11; N, 16.19.

3-(3-Chlorophenyl)-6-(4-nitrophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine (7f)[26]**.** Yield 75%; mp 173–175 °C; IR (KBr) *ν* 3024 (C–H), 1588 (C=N), 1523 (C=C), 1348 (N=O), 1093 (C–N), 1023 (N–N), 779 (C–Cl), 697 (C–S) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.02 (s, 1H, Ar-Hc), 7.53–7.78 (m, 3H, Ar-Hd,e,f), 7.06–7.21 (m, 4H, Ar-H), 4.19 (s, 2H, Ha); MS *m/z* 372 [M^+]; Anal. Calcd for $C_{16}H_{10}ClN_5O_2S$: C, 51.69; H, 2.71; N, 18.84. Found: C, 51.60; H, 2.51; N, 18.72.

3-(3-Chlorophenyl)-6-(4-fluorophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine (7g). Yield 63%; mp 226–228 °C; IR (KBr) *ν* 3019 (C–H), 1597 (C=N), 1531 (C=C), 1163 (C–N), 1007 (N–N), 791 (C–Cl), 713 (C–S) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.19 (s, 1H, Ar-Hc), 7.66–7.85 (m, 3H, Ar-Hd,e,f), 7.31–7.46 (m, 4H, Ar-H), 4.31 (s, 2H, Ha); MS *m/z* 345 [M^+]; Anal. Calcd for $C_{16}H_{10}ClFN_4S$: C, 55.74; H, 2.92; N, 16.25. Found: C, 55.62; H, 2.87; N, 16.19.

3-(3-Chlorophenyl)-6-(4-chlorophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine (7h)[26]**.** Yield 70%; mp 181–182 °C; IR (KBr) *ν* 3047 (C–H), 1617 (C=N), 1493 (C=C), 1149 (C–N), 1034 (N–N), 797 (C–Cl), 719 (C–S) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.13 (s, 1H, Ar-Hc), 7.71–7.83 (m, 3H, Ar-Hd,e,f), 7.27–7.41 (m, 4H, Ar-H), 4.43 (s, 2H, Ha); MS *m/z* 362 [M^+]; Anal. Calcd for $C_{16}H_{10}Cl_2N_4S$: C, 53.20; H, 2.79; N, 15.51. Found: C, 52.96; H, 2.58; N, 15.47.

3-(3-Chlorophenyl)-6-[4-(methylsulfonyl)phenyl]-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine (7i). Yield 61%; mp 190–192 °C; IR (KBr) *ν* 3077 (C–H), 1657 (C=N), 1536 (C=C), 1166 (C–N), 1025 (N–N), 793 (C–Cl), 711 (C–S) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.18 (s, 1H, Ar-Hc), 7.68–7.83 (m, 3H, Ar-Hd,e,f), 7.25–7.38 (m, 4H, Ar-H), 4.30 (s, 2H, Ha), 3.06 (s, 3H, SO_2CH_3); MS *m/z* 405 [M^+]; Anal. Calcd for $C_{17}H_{13}ClN_4O_2S_2$: C, 50.43; H, 3.24; N, 13.84. Found: C, 50.26; H, 3.11; N, 13.61.

3,6-Bis(3-chlorophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine (7j). Yield 68%; mp 217–219 °C; IR (KBr) *ν* 3034 (C–H), 1655 (C=N), 1591 (C=C), 1127 (C–N), 1027 (N–N), 774 (C–Cl), 684 (C–S) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.14 (s, 1H, Ar-Hc), 7.45–7.71 (m, 3H, Ar-Hd,e,f), 6.91–7.20 (m, 4H, Ar-H), 4.27 (s, 2H, Ha); MS *m/z* 361 [M^+]; Anal. Calcd for $C_{16}H_{10}Cl_2N_4S$: C, 53.20; H, 2.79; N, 15.51. Found: C, 53.07; H, 2.60; N, 15.41.

4. Conclusion

In conclusion, we have described efficient and benign synthesis of 1,2,4-triazole systems containing 1,3,4-thiadiazole and 1,3,4-thiadiazines. 1,2,4-Triazole is the key intermediate in the formation of these heterocyclic compounds. Among these, the commonly known systems are generally triazoles fused to pyridines, pyridazines, pyrimidines, pyrazines and triazines. Although there are not many triazoles fused to thiadiazines or thiadiazoles, a number of them are incorporated into a wide variety of therapeutically important compounds possessing a broad spectrum of biological activities. In this connection, we have synthesized thiadiazoles and thiadiazines.

The structure-activity relationship studies revealed that compound **5g** containing NO_2 group at the position 3 of thiadiazoles were active as compared to those with NO_2 group at the position 2. Compounds **5i** and **7g** containing F group at the position 4 in both cases (thiadiazoles and thiadiazines) show potent activities against all bacteria. Compound **7i** containing SO_2CH_3 group at the position 4 exhibited very good antimicrobial activities against *B. megaterium, S. aureus, E. coli* and *P. aeruginosa* as well as antifungal activity against *A. niger.*

5. Acknowledgment

Authors are thankful to Department of Chemistry for providing laboratory facilities. The authors are thankful for facilities and grants given under UGC-SAP for Department Research Support (DRS) and Department of Science & Technology (DST) New Delhi for Fund for Improvement of Science & Technology (FIST). Authors are also thankful to RSIC Chandigarh for providing ^{1}H NMR spectral analysis of the compounds.

6. References

1. L. Labanauskas, V. Kalcas, E. Udrenaite, P. Gaidelis, A. Brukstus, V. Dauksas, *Pharmazie* **2001**, *56*, 617–619.
2. S. Schenone, O. Bruno, A. Ranise, F. Bondavalli, W. Filippelli, G. Falcone, L. Giordano, M. R. Vitelli, *Bioorg. Med. Chem*. **2001**, *9*, 2149–2153.
3. J. J. Baldwin, E. L. Engelhardt, R. Hirschmann, G. S. Ponticello, J. G. Atkinson, B. K. Wasson, C. S. Sweet, A. Scriabine, *J. Med. Chem.* **1980**, *23*, 65–70.
4. A. Varvaresou, A. Tsantili-Kakoulidou, T. Siatra-Papastasikoudi, E. Tiligada, *Arzneimittelforschung* **2000**, *50*, 48–54.
5. A. Varvaresou, T. Siatra-Papastaikoudi, A. Tsotinis, A. Tsantili-Kakoulidou, A. Vamvakides, *Farmaco* **1998**, *53*, 320–326.
6. A. Foroumadi, M. Mirzaei, A. Shafiee, *Pharmazie* **2001**, *56*, 610–612.
7. M. G. Mamolo, V. Falagiani, D. Zampieri, L. Vio, E. Banfi, *Farmaco* **2001**, *56*, 587–592.
8. M. Wujec, M. Pitucha, M. Dobosz, U. Kosikowska, A. Malm, *Acta Pharm.* **2004**, *54*, 251–260.
9. K. Zamani, K. Faghifi, I. Tefighi, M. R. Sharlatzadeh, *Turk. J. Chem*. **2004**, *28*, 95–101.
10. H. Chen, Z. Li, Y. Han, *J. Agric. Food Chem*. **2000**, *48*, 5312–5315.
11. X. J. Zou, G. Y. Jin, Z. X. Zhang, *J. Agric. Food Chem*. **2002**, *50*, 1461–1454.
12. X. J. Zou, L. H. Lai, G. Y. Jin, Z. X. Zhang, *J. Agric. Food Chem*. **2002**, *50*, 3757–3760.
13. F. Clerici, D. Pocar, M. Guido, A. Loche, V. Perlini, M. Brufani, *J. Med. Chem*. **2001**, *44*, 931–936.
14. D. A. Griffin, S. K. Mannion, *Eur. Pat. Appl. Ep* **1986**, 199, 474; *Chem. Abstr*. **1987**, *106*, 98120u.
15. M. I. Hussain, M. Amir, *J. Indian Chem. Soc*. **1986**, *63*, 317–319.
16. S. H. L. Chiu, S. E. W. Huskey, *Drug Metabol. Dispos*. **1998**, *26*, 838–847.
17. H. Emilsson, H. Salender, J. Gaarder, *Eur. J. Med. Chem. Chim. Ther*. **1985**, *21*, 333–338.
18. N. Demirbaş, R. Ugurluoglu, A. Demirbaş, *Bioorg. Med. Chem*. **2002**, *10*, 3717–3723.
19. G. Turan-Zitouni, Z. A. Kaplancikli, K. Erol, F. S. Kiliç, *Farmaco* **1999**, *54*, 218–223.
20. C. S. Reddy, L. S. Rao, A. Nagaraj, *Acta Chim. Slov.* **2010**, *57*, 726–732.
21. M. A. H. A. E. Awas, *Acta Chim. Slov.* **2008**, *55*, 492–501.
22. B. S. Holla, P. M. Akberali, M. K. Shivananda, *Farmaco* **2001**, *56*, 919–927.
23. M. Serwar, T. Akhtar, S. Hameed, K. M. Khan, *Arkivoc* **2009**, (*vii*), 210–221.
24. C. Sridhar, A. C. Bajji, B. M. V. Swamy, *Asian J. Chem.* **2008**, *20*, 1707–1710.
25. A. L. Barry, *The Antimicrobial Susceptibility Test: Principle and Practices,* Illus lea and Febiger (Philadelphia PA, USA), **1976**, p. 180; *Biol. Abstr.* **1976**, *64*, 25183.
26. M. Jag, G. S. R. Anjaneyulu, *Polish J. Chem.* **1987**, *61(4-6)*, 547–555.
27. C. S. Xiong, D. A. John, Z. Han-Zhong, K. Shailaja, C. Gisela, S. N. Sudath, K. W. Edward, *PCT Int. Appl*., WO 2008011045 A2 20080124, **2008**.

Povzetek

Željen obročni sistem 3-(3-klorofenil)-6-aril-5,6-dihidro[1,2,4]triazolo[3,4-*b*][1,3,4]tiadiazolov **5a**–**j** smo pripravili z reakcijo med 4-amino-5-(3-klorofenil)-4*H*-1,2,4-triazol-3-tiolom in različnimi aril aldehidi v prisotnosti katalitskih količin *p*-TsOH v suhem DMF; 3-(3-klorofenil)-6-aril-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]tiadiazine **7a**–**j** pa smo pripravili z uporabo 4-amino-5-(3-klorofenil)-4*H*-1,2,4-triazol-3-tiola in različnih fenacil bromidov v suhem metanolu. Njihovi IR, ^{1}H NMR in masni spektroskopski podatki ter elementne analize so bili v skladu s predvidenimi strukturami. Za vse novopripravljene spojine smo določili antimikrobne aktivnosti. Nekatere izmed teh spojin kažejo opazno večje zaviranje rasti bakterij in gliv v primerjavi s standardnimi zdravili.