

Facile Synthesis and Antimicrobial Activities of 5H-[1,2,4]triazol[1,3,4]thiadiazine Derivatives

G. S. KALWANIA*, SAVITA CHOUDHARY and S. CHOMAL

Department of Chemistry, S. K. Govt. P.G. College (University of Rajasthan), Sikar, India
gskalwania@gmail.com

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Abstract: Structurally diverse new series of 5H-[1,2,4]triazolo[3,4b] [1,3,4]thiadiazine heterocycles having medicinally privileged nucleus have been synthesized by simple and solvent free environmental benign methods. A mixture of 4-amino-5-substituted-1,2,4-triazolo-3-thiol and β -diketone/ β -ketoester and catalytic amount of hydrazine hydrate exposed to microwave irradiation in microwave oven. The intermediate 4-amino-1,2,4-triazole-3-thiols were also synthesized by entirely new solvent free environmental friendly methods. The synthesized target compounds (**4a-o**) were characterized by elemental analysis as well as spectral studies and screened for antimicrobial activities.

Keywords: 1,2,4-Triazole, 1,4-Thiazine, 1,3,4-Thiadiazine, Biodynamic nucleus, β -Ketoester, Irradiation, Antimicrobial, Microwave.

Introduction

Synthesis of privileged class of heterocycles has become one of the prime areas of research in the field of synthetic and medicinal chemistry. In general, biological active compounds are derived from heterocyclic structures. Heterocycles containing 1,2,4-triazole and 1,3,4-thiadiazine nucleus have been well studied for a number of pathological conditions including inflammation^{1,2}, hypertension³ and aching⁴. The 1,3,4-thiadiazine derivatives in which 1,4-thiazines fused with 1,2,4-triazole nucleus are important scaffold in several natural and synthetic compounds of significant pharmacological properties and also have structural similarity with biologically most active phenothiazines in having fold along N-S axis⁵. These heterocycles have diverse applications as powerful antibacterial⁶⁻⁹, anti-mycobacterial^{10,11}, antimitotic^{12,13}, antifungal^{14,15}, antidepressant^{16,17}, analgesic¹⁸, anti-inflammatory, anticonvulsant¹⁹, antihypertensive²⁰, antitumoral^{21,22}, antiviral²³, antiplatelet²⁴ and antithrombotic²⁵.

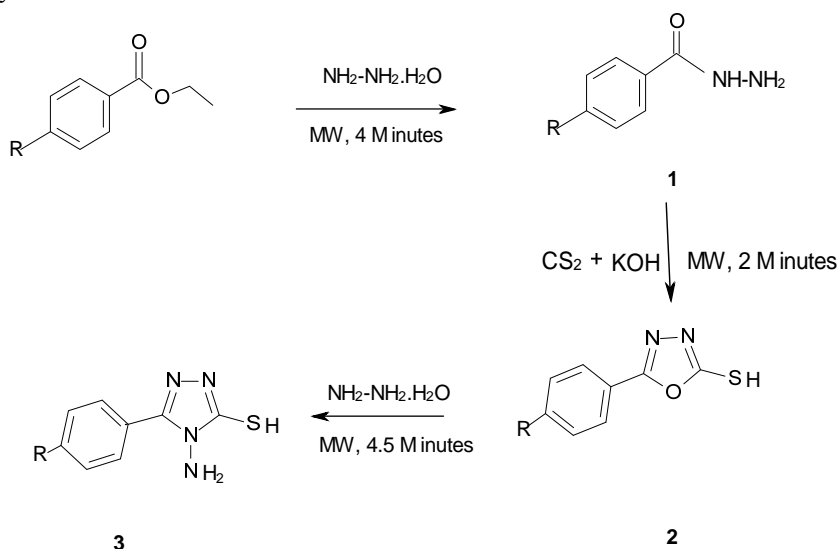
In view of these diverse biological importance of these heterocycles, a number of hitherto unknown 1,3,4-thiadiazine derivatives containing 1,2,4-triazole nucleus were synthesized by environmental benign solvent free method, characterized and screened for their biological activities to explore potent biologically active molecules.

Experimental

Melting points of all the synthesized compounds were determined on open aluminum block and are uncorrected. Purity was checked by thin layer chromatography using Merck silica gel G-60. IR spectra were recorded in KBr on Shimadzu Affinity-1 FTIR spectrophotometer. ^1H NMR spectra were recorded on Varian Gemini 400 spectrometer (300 MHz) using TMS as an internal standard. The mass spectra were recorded using Jeol SX 102 spectrometer at 70 eV.

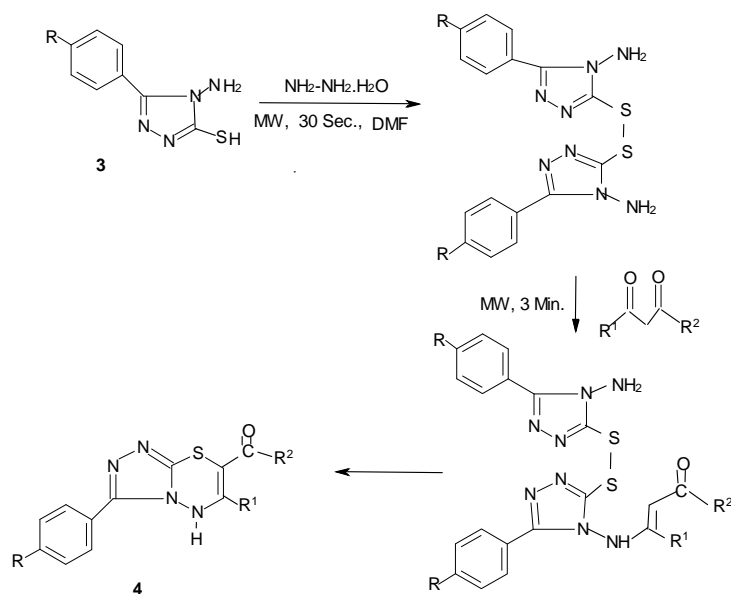
Results and Discussion

Most attention have been paid to synthesize 1,4-thiazine derivatives fused with 1,2,4-triazole nucleus which gives ultimately new series of 1,3,4-thiadiazine derivatives. The intermediate 4-amino-5-substituted-1,2,4-triazole-3-thiols (**3**) were prepared in three steps by new environmentally benign solvent free methods (Scheme 1) using microwave irradiation as well as by conventional methods²⁶.



Scheme 1

The conventional methods for synthesis of these intermediates require hazardous chemicals like pyridine and long heating systems. The yield obtained from microwave methods was better as compared to conventional method. The target compounds, 5*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine derivatives (**4a-o**) were prepared by environmental friendly method (Scheme 2) in which the mixture of intermediate compound **3**, β -diketone / β -ketoester and catalytic amount of hydrazine hydrate irradiated in microwave oven. The purity of the compounds was checked by TLC. The synthesized compounds were characterized with the help of spectral data (IR, ^1H NMR, and Mass) and elemental analysis. All data were found in accordance with the proposed structures of synthesized compounds. All the 5*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine derivatives (**4a-o**) along with some standard antibiotics and antifungal drugs were screened for their antimicrobial activities against selected bacteria and fungus. All the antimicrobial activity results are summarized in Table 1. Fluoro substituted thiadiazine derivatives **4b**, **4g**, **4l** and nitro derivatives **4e**, **4j**, **4o** were found moderately active against selected bacteria and fungi. However, the other compounds were also active but not at satisfactory level.



Scheme 2

Table 1. Antimicrobial activities of 5*H*-[1,2,4]triazolo[3,4*b*][1,3,4]thiadiazines derivatives (**4a-o**)

Compd.	Zone of Inhibition in mm					
	Antibacterial Activity				Antifungal Activity	
	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>B. megaterium</i>	<i>A. niger</i>	<i>C. albicans</i>
4a	10.00	12.25	13.00	11.50	9.50	9.00
4b	16.00	17.25	18.00	16.25	14.50	16.50
4c	13.50	12.50	13.00	12.00	11.50	11.00
4d	12.50	13.00	12.50	11.75	10.00	10.75
4e	15.00	15.50	16.00	15.00	13.00	14.75
4f	11.20	10.75	11.75	10.50	9.00	9.25
4g	17.00	18.00	19.00	17.75	15.75	16.00
4h	13.00	14.25	15.25	14.75	12.00	11.75
4i	11.50	12.75	12.00	12.25	11.00	11.25
4j	16.50	16.00	16.75	15.50	13.00	12.75
4k	10.75	10.75	11.50	11.25	9.75	10.00
4l	18.00	18.00	19.00	18.25	16.25	16.00
4m	14.25	14.25	14.00	13.75	10.50	11.25
4n	12.50	13.00	13.25	13.50	10.75	10.25
4o	15.75	15.50	16.00	17.00	14.00	13.75
Amoxocillin	20.25	21.50	22.50	20.75	-	-
Ciprofloxacin	23.50	22.25	24.00	20.75	-	-
Griseofulvin	-	-	-	-	24.00	22.75

Synthesis of arylhydrazides (1)

Conventional method

To the esters of substituted aromatic acids (10 mmol), hydrazine hydrate (10 mmol) was added and refluxed the solution for 30 min. To the refluxing mixture, 20 mL of ethanol was added as a solvent in order to homogenize the contents. The resulting mixture was further allowed to reflux for 6 h. Excess ethanol was distilled out and the contents were allowed to cool. The crystals formed were filtered, washed thoroughly with water and dried.

Microwave irradiation method

A mixture of esters of substituted aromatic acids (10 mmol) and hydrazine hydrate (10 mmol) exposed to microwave irradiations intermittently at 30 seconds for four minutes in microwave oven. After completion of reaction, the contents were allowed to cool at room temperature. The crystals separated out were filtered, washed thoroughly with water and dried to get aryl hydrazide (1)

Synthesis of 5-(4-Substitutedphenyl)-1,3,4-oxadiazole-2-thiols (2)

Conventional Method

To a solution of aryl hydrazide **1** (10 mmol) in ethanol (30 mL), potassium hydroxide (10 mmol) in absolute ethanol (50 mL) and carbon disulfide (20 mmol) were added and refluxed for about 5 h till evolution of hydrogen sulfide was ceased. The reaction mixture was cooled at room temperature and diluted with water. On acidification with dilute hydrochloric acid, the required oxadiazoles derivative was precipitated. It was filtered, washed thoroughly with cold water and recrystallized from ethanol.

Microwave irradiation method

A mixture of aryl hydrazide **1** (10 mmol) and potassium hydroxide (10 mmol) was well grinded to make a fine homogeneous powder. To this powder carbondisulfide (20 mmol) was added and exposed to microwave irradiations intermittently at 20 seconds for two minutes. The reaction mixture was cooled and neutralized with dilute hydrochloric acid. The solid separated out on neutralization was filtered, washed with cold water and dried. There is no need to purify the product 1,3,4-oxadiazole-2-thiol derivatives (**2**) for further reaction (Scheme 1).

Synthesis of 4-amino-1,2,4-triazole-3-thiols (3)

Conventional method

A mixture of compound **2** (10 mmol) and hydrazine hydrate (10 mmol) in dry pyridine (15 mL) was refluxed for about 4 h. The reaction mixture was cooled at room temperature and neutralized with dilute hydrochloric acid. The solid precipitated out was filtered, washed thoroughly with cold water and recrystallized from ethanol.

Microwave irradiation method

A mixture of compound **2** (10 mmol) and hydrazine hydrate (10 mmol) was exposed to microwave irradiations intermittently at 30 seconds for 4.5 minutes. After completion of reaction, the hot reaction mixture was cooled at room temperature and neutralized with dilute hydrochloric acid. The solid separated out was filtered, washed thoroughly with water and crystallized from ethanol (Scheme 1).

4-Amino-5-(4-methoxyphenyl)-1,2,4-triazole-3-thiol: Yield- 68%; m. p. 204 °C

4-Amino-5-(4-fluorophenyl)-1,2,4-triazole-3-thiol: Yield- 65%; m. p. 139 °C

4-Amino-5-(4-chlorophenyl)-1,2,4-triazole-3-thiol: Yield-70%; m. p. 212 °C

4-Amino-5-(4-bromophenyl)-1,2,4-triazole-3-thiol: Yield-70%; m. p. 205 °C

4-Amino-5-(4-nitrophenyl)-1,2,4-triazole-3-thiol: Yield- 62%; m. p. 213 °C

Synthesis of 5H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazines (4a-o)

A mixture of compound **3** (10 mmol), catalytic amount of hydrazine hydrate (1 mmol) and DMF (5 mL) as an energy transfer medium was exposed to microwave irradiations for 30 seconds. After that β -diketone/ β -ketoester (10 mmol) was added to the reaction mixture and again exposed to microwave irradiations intermittently at 30 seconds for three minutes. After completion of reaction as monitored by TLC, the reaction mixture was cooled and transferred to crushed ice. The solid separated out was filtered, washed with 50% ethanol and crystallized from ethanol to get pure product **4a-o** (scheme 2). All the physical and analytical data are summarized below

7-Ethoxycarbonyl-3-(4-methoxyphenyl)-6-methyl-5H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazines (4a)

Yield 65%, m.p. 135 °C; IR (KBr, ν_{\max} , cm^{-1}): 3380 (N-H), 1225, 1030 (C-O-C), 2990 (C-H aliphatic), 3070 (C-H aromatic), 1597 (C=N), 1680 ($>\text{C}=\text{O}$), 1058 (N-N); ^1H NMR (CDCl_3 , δ): 7.2-7.9 (m, 4H, Ar-H), 8.7 (s, 1H, N-H), 2.20 (s, 3H, CH_3 at C_6), 3.8 (s, 3H, OCH_3 at C_7), 4.10 (q, 2H, CH_2 at C_7), 1.20 (t, 3H, CH_3 at C_7); Mass m/z 332 [M^+], 331 [M-H], 303[M- C_2H_5], 304 [M- C_2H_4], 287 [M- OC_2H_5], 285 [M- $\text{C}_2\text{H}_5\text{OH}+\text{CO}$], 225 [M- $\text{C}_6\text{H}_4\text{OCH}_3$], 107 [$\text{C}_6\text{H}_4\text{OCH}_3$]; Anal. Calculated (%) for $\text{C}_{15}\text{H}_{16}\text{N}_4\text{O}_3\text{S}$: C, 54.21; H, 4.81; N, 16.86. Found (%): C, 53.95; H, 4.76; N, 16.79.

7-Ethoxycarbonyl-3-(4-fluorophenyl)-6-methyl-5H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (4b)

Yield 60%, m. p. 132°C; IR (KBr, ν_{\max} , cm^{-1}): 3385 (N-H), 1230, 1035 (C-O-C), 2995 (C-H aliphatic), 3050 (C-H aromatic), 1588 (C=N), 1675 ($>\text{C}=\text{O}$), 1055 (N-N); ^1H NMR (CDCl_3 , δ): 7.8-8.5 (m, 4H,Ar-H), 8.75 (s, 1H, N-H), 2.2 (s, 3H, CH_3 at C_6), 4.05 (q, 2H, CH_2 at C_7), 1.15 (t, 3H, CH_3 at C_7); Mass m/z 320 [M^+], 319 [M-H], 291 [M- C_2H_5], 292 [M- C_2H_4], 275 [M- OC_2H_5], 247 [M- $\text{C}_2\text{H}_5\text{OH}+\text{CO}$], 225 [M- $\text{C}_6\text{H}_4\text{F}$], 95 [$\text{C}_6\text{H}_5\text{F}$]; Anal. Calculated (%) for $\text{C}_{14}\text{H}_{13}\text{FN}_4\text{O}_2\text{S}$: C, 52.5; H, 4.1; N, 17.5. Found (%):C, 52.1; H, 3.95; N, 17.31.

3-(4-Chlorophenyl)-7-ethoxycarbonyl-6-methyl-5H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (4c)

Yield 66%, m. p. 129°C; IR (KBr, ν_{\max} , cm^{-1}): 3380 (N-H), 1220, 1030 (C-O-C), 2990 (C-H aliphatic), 3060 (C-H aromatic), 1590 (C=N), 1675 ($>\text{C}=\text{O}$), 740 (C-Cl), 1043 (N-N); ^1H NMR (CDCl_3 , δ): 7.6-8.1 (m, 4H,Ar-H.), 8.7 (s, 1H, N-H), 2.25 (s, 3H, CH_3 at C_6), 4.0 (q, 2H, CH_2 at C_7), 1.25 (t, 3H, CH_3 at C_7); Mass m/z 336 [M^+] 335 [M-H], 307 [M- C_2H_5], 308 [M- C_2H_4], 291[M- OC_2H_5], 262 [$\text{C}_2\text{H}_5\text{OH}+\text{CO}$], 35 [Cl], 255 [M- $\text{C}_6\text{H}_4\text{Cl}$], 111 [$\text{C}_6\text{H}_4\text{Cl}$]; Anal. Calculated (%) for $\text{C}_{14}\text{H}_{13}\text{ClN}_4\text{O}_2\text{S}$: C, 49.9; H,3.8; N,16.6. Found (%): C, 50.52; H, 3.5; N, 16.2.

3-(4-Bromophenyl)-7-ethoxycarbonyl-6-methyl-5H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (4d)

Yield 70%, m.p. 139°C; IR (KBr, ν_{\max} , cm^{-1}): 3340 (N-H), 1230, 1035 (C-O-C), 2995 (C-H aliphatic), 3070 (C-H aromatic), 1593 (C=N), 1665 ($>\text{C}=\text{O}$), 1060 (N-N), ^1H NMR (CDCl_3 , δ):

7.3-7.9 (m, 4H, Ar-H_s), 8.65 (s, 1H, N-H), 2.15 (s, 3H, CH₃ at C₆), 4.15 (q, 2H, CH₂ at C₇), 1.25 (t, 3H, CH₃ at C₇); Anal. Calculated (%) for C₁₄H₁₃BrN₄O₂S: C, 44.0; H, 3.4; N, 14.6. Found (%): C, 43.84; H, 3.21; N, 14.35.

7-Ethoxycarbonyl-6-methyl-3-(4-nitrophenyl)-5H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (4e)

Yield 63%, m. p. 124°C; IR (KBr, ν_{\max} , cm⁻¹): 3370 (N-H), 1230, 1040 (C-O-C), 3070 (C-H aromatic), 2990 (C-H aliphatic), 1590 (C=N), 1680 (>C=O), 1060 (N-N); ¹H NMR (CDCl₃, δ): 7.45-8.25 (m, 4H, Ar-H_s), 8.5 (s, 1H, N-H), 2.20 (s, 3H, CH₃ at C₆), 4.05 (q, 2H, CH₂ at C₇), 1.15 (t, 3H, CH₃ at C₇); Anal. Calculated (%) for C₁₄H₁₃N₅O₄S: C, 48.41; H, 3.74; N, 20.17. Found (%): C, 48.34; H, 3.71; N, 20.14.

7-Ethoxycarbonyl-3-(4-methoxyphenyl)-6-phenyl-5H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazines (4f)

Yield 65%, m. p. 141 °C; IR (KBr, ν_{\max} , cm⁻¹): 3385 (N-H), 1235, 1040 (C-O-C), 2998 (C-H aliphatic), 3090 (C-H aromatic), 1595 (C=N), 1670 (>C=O), 1065 (N-N); ¹H NMR (CDCl₃, δ): 7.8-8.2 (m, 10H, Ar-H), 8.8 (s, 1H, N-H), 3.75 (s, 4H, OCH₃ at 4'), 4.1 (q, 2H, CH₂ at C₇), 1.18 (t, 3H, CH₃ at C₇); Mass m/z 394 [M⁺], 393 [M-H], 365 [M-C₂H₅], 366 [M-C₂H₄], 349 [M-OC₂H₅], 320 [C₂H₅OH+CO], 317 [M-C₆H₅], 77 [C₆H₅], 287 [M-C₆H₄OCH₃], 107 [C₆H₄OCH₃]; Anal. Calculated (%) for C₂₀H₁₈N₄O₃S: C, 60.91; H, 4.56; N, 14.21. Found (%): C, 60.78; H, 4.52; N, 14.16.

7-Ethoxycarbonyl-3-(4-fluorophenyl)-6-phenyl-5H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (4g)

Yield 62%, m. p. 138°C; IR (KBr, ν_{\max} , cm⁻¹): 3385 (N-H), 1240, 1045 (C-O-C), 3090 (C-H aromatic), 2985 (C-H aliphatic), 1610 (C=N), 1685 (>C=O), 1075 (N-N); ¹H NMR (CDCl₃, δ): 8.0-8.7 (m, 9H, Ar-H), 8.75 (s, 1H, N-H), 4.10 (q, 2H, CH₂ at C₇), 1.20 (t, 3H, CH₃ at C₇); Mass m/z 382 [M⁺], 381 [M-H], 353 [M-C₂H₅], 354 [C₂H₄], 337 [OC₂H₅], 308 [C₂H₅OH], 305 [M-C₆H₅], 77 [C₆H₅], 287 [M-C₆H₄F], 95 [C₆H₄F]; Anal. Calculated (%) for C₁₉H₁₅FN₄O₂S: C, 59.5; H, 3.9; N, 14.6. Found (%): C, 59.32; H, 3.78; N, 14.49.

3-(4-Chlorophenyl)-7-ethoxycarbonyl-6-phenyl-5H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (4h)

Yield 67%, m. p. 134°C; IR (KBr, ν_{\max} , cm⁻¹): 3390 (N-H), 1245, 1035 (C-O-C), 3045 (C-H aromatic), 2980 (C-H aliphatic), 1605 (C=N), 1685 (>C=O), 1060 (N-N), 765 (C-Cl); ¹H NMR (CDCl₃, δ): 7.7-8.2 (m, 9H, Ar-H), 8.70 (s, 1H, N-H), 4.05 (q, 2H, CH₂ at C₇), 1.15 (t, 3H, CH₃ at C₇); Mass m/z 398 [M⁺], 397 [M-H], 369 [M-C₂H₅], 370 [M-C₂H₅], 353 [M-OC₂H₅], 324 [M-C₂H₅OH+CO], 321 [M-C₆H₅], 77 [C₆H₅], 287 [M-C₆H₄Cl], 111 [C₆H₄Cl]; Anal. Calculated (%) for C₁₉H₁₅ClN₄O₂S: C, 57.5; H, 3.7; N, 14.0. Found (%): C, 56.98; H, 3.6; N, 13.89.

3-(4-Bromophenyl)-7-ethoxycarbonyl-6-phenyl-5H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (4i)

Yield 69%, m. p. 145°C; IR (KBr, ν_{\max} , cm⁻¹): 3390 (N-H), 1240, 1035 (C-O-C), 3080 (C-H aromatic), 2985 (C-H aliphatic), 1598 (C=N), 1665 (>C=O), 1068 (N-N); ¹H NMR (CDCl₃, δ): 7.4-6.98 (m, 9H, Ar-H), 8.75 (s, 1H, N-H), 4.10 (q, 2H, CH₂ at C₇), 1.15 (t, 3H, CH₃ at C₇); Anal. Calculated (%) for C₁₉H₁₅BrN₄O₂S: C, 51.4; H, 3.3; N, 12.6. Found (%): C, 51.05; H, 3.12; N, 12.48.

7-Ethoxycarbonyl-3-(4-nitrophenyl)-6-phenyl-5H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (4j)

Yield 63%, m. p. 151°C; IR (KBr, $\nu_{\max.}$, cm^{-1}): 3260 (N-H), 1240, 1035 (C-O-C), 3085 (C-H aromatic), 2985 (C-H aliphatic), 1585 (C=N), 1675 ($>\text{C}=\text{O}$), 1065 (N-N); ^1H NMR (CDCl_3 , δ): 7.5-8.2 (m, 9H, Ar-H), 8.65 (s, 1H, N-H), 4.0 (q, 2H, CH_2 at C_7), 1.20 (t, 3H, CH_3 at C_7); Anal. Calculated (%) for $\text{C}_{19}\text{H}_{15}\text{N}_5\text{O}_4\text{S}$: C, 55.74; H, 3.66; N, 17.11. Found (%): C, 55.68; 3.64; N, 17.04.

3-(4-Methoxyphenyl)-6-methyl-7-methylcarbonyl-5H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazines (4k)

Yield 64%, m. p. 122°C; IR (KBr, $\nu_{\max.}$, cm^{-1}): 3390 (N-H), 2970 (C-H aliphatic), 3080 (C-H aromatic), 1590 (C=N), 1660 ($>\text{C}=\text{O}$), 1060 (N-N); ^1H NMR (CDCl_3 , δ): 7.8-8.5 (m, 4H, Ar-H), 8.7 (s, 1H, N-H), 2.15 (s, 3H, CH_3 at C_6), 3.8 (s, 3H, OCH_3 at $4'$), 3.5 (s, 3H, COCH_3 at C_7); Mass m/z 302 [M^+], 301 [$\text{M}-\text{H}$], 287 [$\text{M}-\text{CH}_3$], 259 [$\text{M}-\text{COCH}_3$], 242 [$\text{M}-\text{CH}_3\text{OH}+\text{CO}$], 195 [$\text{M}-\text{C}_6\text{H}_4\text{OCH}_3$], 107 [$\text{C}_6\text{H}_4\text{OCH}_3$]; Anal. Calculated (%) for $\text{C}_{14}\text{H}_{14}\text{N}_4\text{O}_2\text{S}$: C, 55.6; H, 4.63; N, 18.54. Found (%): C, 55.54; H, 4.59; N, 18.48.

3-(4-Fluorophenyl)-6-methyl-7-methylcarbonyl-5H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (4l)

Yield 62%, m. p. 114°C; IR (KBr, $\nu_{\max.}$, cm^{-1}): 3380 (N-H), 2995 (C-H aliphatic), 3065 (C-H aromatic), 1590 (C=N), 1650 ($>\text{C}=\text{O}$), 1070 (N-N); ^1H NMR (CDCl_3 , δ): 7.9-7.5 (m, 4H, Ar-H), 8.7 (s, 1H, N-H), 2.20 (s, 3H, CH_3 at C_6), 3.6 (s, 3H, COCH_3 at C_7); Mass m/z 290 [M^+], 289 [$\text{M}-\text{H}$], 275 [$\text{M}-\text{CH}_3$], 247 [$\text{M}-\text{COCH}_3$], 230 [$\text{M}-\text{CH}_3\text{OH}+\text{CO}$], 195 [$\text{C}_6\text{H}_4\text{F}$], 95 [$\text{C}_6\text{H}_4\text{F}$]; Anal. Calculated (%) for $\text{C}_{13}\text{H}_{11}\text{FN}_4\text{OS}$: C, 53.7; H, 3.7; N, 19.3. Found (%): C, 53.59; H, 3.62; N, 19.12.

3-(4-Chlorophenyl)-6-methyl-7-methylcarbonyl-5H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (4m)

Yield 65%, m. p. 119°C; IR (KBr, $\nu_{\max.}$, cm^{-1}): 3370 (N-H), 2985 (C-H aliphatic), 3065 (C-H aromatic), 1595 (C=N), 1660 ($>\text{C}=\text{O}$), 1065 (N-N); ^1H NMR (CDCl_3 , δ): 7.7-8.3 (m, 4H, Ar-H), 8.8 (s, 1H, N-H), 2.15 (s, 3H, CH_3 at C_6), 3.45 (s, 3H, COCH_3 at C_7); Anal. Calculated (%) for $\text{C}_{13}\text{H}_{11}\text{ClN}_4\text{OS}$: C, 50.8; H, 3.5; N, 18.2. Found (%): C, 50.68; H, 3.43; N, 18.13.

3-(4-Bromophenyl)-6-methyl-7-methylcarbonyl-5H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (4n)

Yield 6%, m. p. 135°C; IR (KBr, $\nu_{\max.}$, cm^{-1}): 3385 (N-H), 2985 (C-H aliphatic), 3075 (C-H aromatic), 1598 (C=N), 1655 ($>\text{C}=\text{O}$), 1065 (N-N); ^1H NMR (CDCl_3 , δ): 7.6-8.3 (m, 4H, Ar-H), 8.7 (s, 1H, N-H), 2.25 (s, 3H, CH_3 at C_6), 3.4 (s, 3H, COCH_3 at C_7); Mass m/z 351 [M^+], 350 [$\text{M}-\text{H}$], 336 [$\text{M}-\text{CH}_3$], 308 [COCH_3], 291 [$\text{M}-\text{CH}_3\text{OH}+\text{CO}$], 195 [$\text{M}-\text{C}_6\text{H}_4\text{Br}$], 156 [$\text{C}_6\text{H}_4\text{Br}$], 272 [$\text{M}-\text{Br}$], 79 [Br]; Anal. Calculated (%) for $\text{C}_{13}\text{H}_{11}\text{BrN}_4\text{OS}$: C, 44.4; H, 3.1; N, 15.9. Found (%): C, 44.15; H, 2.98; N, 15.81.

6-Methyl-7-methylcarbonyl-3-(4-nitrophenyl)-5H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (4o)

Yield 67%, m. p. 128 °C; IR (KBr, $\nu_{\max.}$, cm^{-1}): 3370 (N-H), 3070 (C-H aromatic), 2990 (C-H aliphatic), 1580 (C=N), 1665 ($>\text{C}=\text{O}$), 1050 (N-N); ^1H NMR (CDCl_3 , δ): 7.60-8.30 (m, 4H, Ar-H), 8.5 (s, 1H, N-H), 2.20 (s, 3H, CH_3 at C_6), 3.45 (s, 3H, COCH_3 at C_7);

Mass m/z 317 [M⁺], 316 [M-H], 302 [M-CH₃], 274 [M-COCH₃], 257 [M-CH₃OH+CO], 195 [M-C₆H₄NO₂], 122 [C₆H₄NO₂], 271 [M-NO₂], 287 [M-NO]; Anal. Calculated (%) for C₁₃H₁₁N₅O₃S: C,49.21; H,3.47; N,22.08. Found (%): C,49.12; H,3.44; N,21.02

Antimicrobial activity

All the synthesized thiadiazines derivatives evaluated for antimicrobial activities *in vitro* using agar-plate diffusion technique¹⁸ by measuring the zone of inhibition in mm. The antibacterial activity was evaluated against bacteria *Escherichia coli* ATCC25922 (*E. coli*), *Pseudomonas aeruginosa* ATCC 27853 (*P. aeruginosa*), *Staphylococcus aureus* ATCC 25923 (*S. aureus*) and *Bacillus megaterium* ATCC 14518 (*B. megaterium*) at 40 µg/mL concentration of samples with standard drugs amoxicillin and ciprofloxacin. After completion of incubation period, the zone of inhibition of growth in the form of diameter in mm was measured (Table 1).

Antifungal activities was evaluated against *Aspergillus neiger* (*A. neiger*) and *canadida albicans* (*C. albicans*) at 40 µg/mL concentration of samples using Griseofulvin as standard drug. After completion of the incubation period, the zone of inhibition of growth in the form of diameter in mm was measured (Table 1).

Conclusion

We have developed environmental friendly synthetic method for synthesis of novel heterocycles having fused 1,2,4-triazole and 1,3,4-thiadiazine nucleus. All the key intermediates and target compounds were synthesized by altogether new solvent free methods using microwave irradiations. The 1,2,4-triazolo[3,4-b][1,3,4]thiadiazine derivatives possess a broad spectrum of biological activities. The structure antimicrobial activity relationship studies revealed that fluoro and nitrothiadiazine derivatives were moderately active against selected bacteria and fungi but other compounds were not at satisfactory level. The target thiadiazine derivatives were characterized by elemental analysis and spectral studies.

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