

# Synthesis and biological activity of 1,2,4-triazolo-[3,4-b]thiadiazole as antimicrobial agents

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## ABSTRACT

Some novel 6-fluoro chroman derivatives having 1,2,4-triazolo-[3,4-b]thiadiazole were synthesized and characterized by IR, NMR and mass spectral analysis. All synthesized compounds were screened for antimicrobial activity using broth dilution method. All the compounds showed good antimicrobial activity and compound 5e showed significant antibacterial activity.

**Keywords:** 6-Fluoro chroman; 1,2,4-triazole; triazolothiadiazole; antimicrobial activity

## 1. INTRODUCTION

Over the years, synthetic heterocyclic chemistry is providing momentum to the development of new drug scaffolds through interactive manipulation of functional groups around the basic skeleton. Among these, heterocyclic compounds have been given special importance because of a wide variety of biological properties associated with them. The importance of heterocycles in biological systems encouraged chemists to design and modify new heterocyclic compounds [1,2]. During the last two decades, the chemistry of 1,2,4-triazole and 1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazole and their derivatives have received considerable attention owing to their synthetic and effective biological importance [3-5]. 1,2,4-Triazoles and their derivatives occupy an essential position in medicinal chemistry because of their potential biological activities such as antibacterial [6], antifungal [7], anti-tubercular [8], anti-inflammatory [9] etc. The 1,2,4-triazole ring is an integral part found in various drugs such as rizatriptan, ribavirin, and fluconazole (Fig.1), which find a wide range of applications in pharmaceutical industry [10-12].

Our previous lab members have synthesized 2-(3'5'-dichlorobenzo[b]thiophen-2'-yl)-5-arylamino-1,3,4-thiadiazoles [13] from triazole and some new thiosemicarbazide and 1,3,4-thiadiazole heterocycles bearing the benzo[b]thiophene nucleus [14] as potent antitubercular and antimicrobial agents. In light of wide varieties of therapeutic activities exhibited by thiadiazole, we have embarked upon the synthesis of some new thiadiazole derivatives which have been described in following sections.

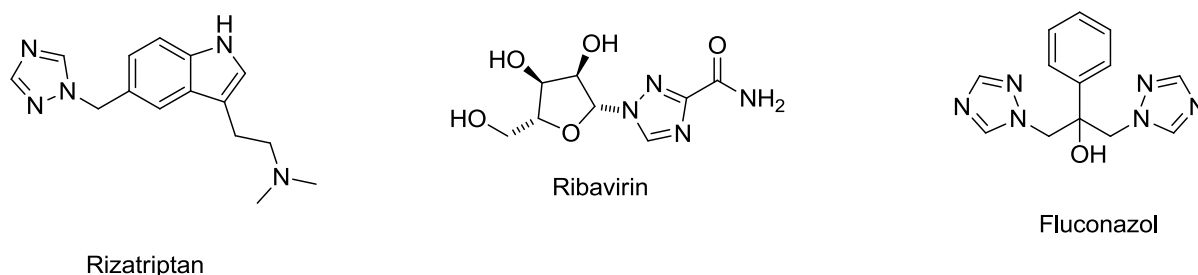


Fig.1

## 2. EXPERIMENTAL

All chemicals and solvents were purchased from Spectrochem Pvt Ltd., Mumbai of LR grade and were used without further purification. Melting points were taken in open capillary method and are uncorrected. IR spectra were recorded on FTIR-8400 spectrophotometer (Shimadzu, Kyoto, Japan), using DRS probe KBr pallet.  $^1\text{H}$ NMR spectra of the synthesized compounds were recorded on a Bruker-Avance-II (400 MHz)  $\text{CDCl}_3$  and  $\text{DMSO-d}_6$  solvent. Chemical shifts are expressed in  $\delta$  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).

To a stirred solution of methyl 6-fluorochroman-2-carboxylate **1**, (2.0 g, 0.01 mol) in absolute ethanol (25 ml) cooled at  $(-5)^\circ\text{C}$ , hydrazine hydrate (99 %), (4.0 ml, 0.08 mol) was added and reaction mixture was allowed to stir at  $0(-5)^\circ\text{C}$  for 10 hours. After the completion of reaction solid residue obtained was filtered, washed with cold ethanol and dried to afford 6-fluorochroman-2-carbohydrazide **2**, Yield: 2.0 g (98 %).

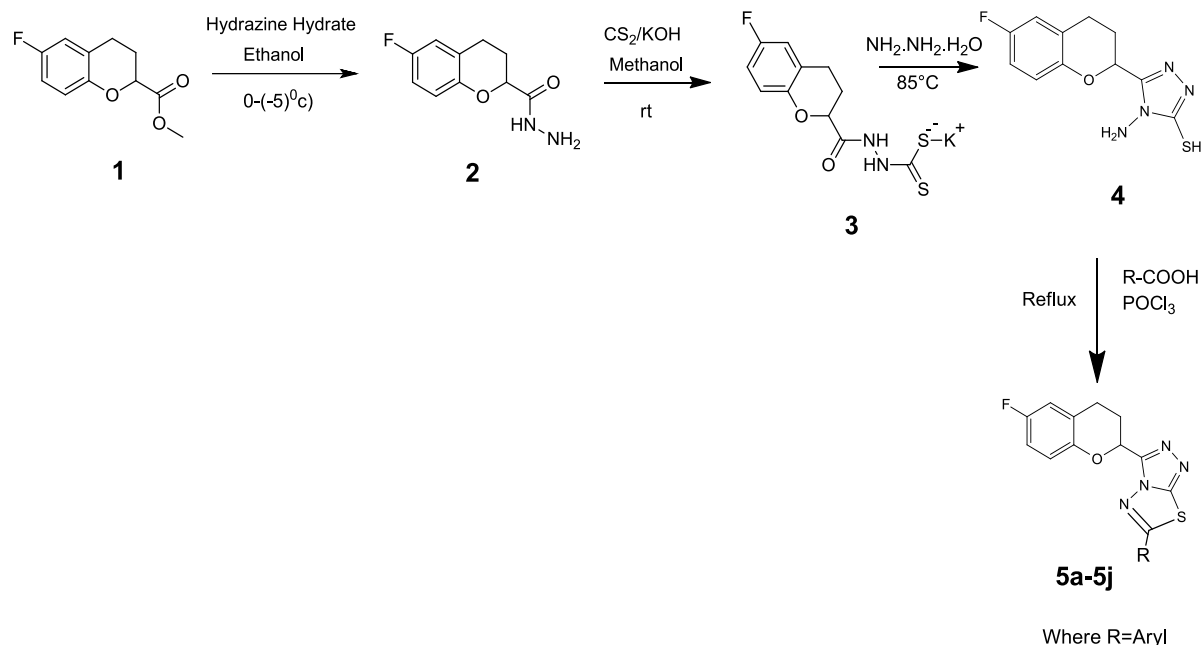
To a stirred solution 6-fluorochroman-2-carbohydrazide **2** (2.0 g, 0.1 mol) and potassium hydroxide (1.0g, 0.15 mol) in methanol (25 ml), carbon disulphide (11.4 g, 0.15 mol) was added. Reaction mixture was allowed to stir for 22-24 hours at RT. After completion of reaction precipitate obtained was filtered, washed with diethyl ether and dried to afford potassium 2-[(6-fluorochroman-2-yl) carbonyl] hydrazine carbodithioate **3**, Yield: 2.88 g (93 %). There is no need to purify the salt for further reaction.

A mixture of potassium 2-[(6-fluorochroman-2-yl) carbonyl] hydrazine carbodithioate **3** (3.5 g, 0.1 M) in water (5 ml) and hydrazine hydrate (3.4 ml, 0.05 M) was refluxed for 6-7 h with occasional shaking. The colour of the reaction mixture changed to green with the evolution of hydrogen sulfide. A homogenous reaction mixture was obtained during the reaction process. The completion of the reaction was monitored on TLC. The reaction mixture was cooled to room temperature and diluted with water (100 ml). On acidification with concentrated hydrochloric acid, the required triazole **4** gets precipitated. Further it was filtered, washed thoroughly with cold water and recrystallized from ethanol.

### 2. 1. General procedure for the preparation of 3-(6-Fluorochroman-2-yl)-6-aryl [1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles.

A mixture of 4-amino-5-(6-fluorochroman-2-yl)-4*H*-1,2,4-triazole-3-thiol (2.66 g, 0.01 mol) and different aryl acids (0.01 mol) in phosphorous oxychloride (15 ml) was refluxed with continuous stirring. After completion the reaction (15-16 hours monitoring by TLC), the content was cooled to room temperature and was poured on crushed ice and thus solid

separated out was filtered, washed with water and neutralized with sodium bicarbonate solution. Crude product was purified by column chromatography to give the analytical pure compounds. Physical constants of newly synthesized triazolo[3,4-b][1,3,4]thiadiazoles derivatives 5a-5j are recorded in Table 1.



**Table 1.** Physical Constant table of 1,2,4-triazolo-[3,4-b]thiadiazole derivatives (5a-5j).

Sr. No	Compound	Substitution R	M. F.	M. W.	Yield (%)
1	5a	3-Cl C <sub>6</sub> H <sub>4</sub>	C <sub>18</sub> H <sub>12</sub> ClFN <sub>4</sub> OS	386.83	95
2	5b	3,4-diOMe C <sub>6</sub> H <sub>3</sub>	C <sub>20</sub> H <sub>17</sub> FN <sub>4</sub> O <sub>3</sub> S	412.43	79
3	5c	4-NH <sub>2</sub> C <sub>6</sub> H <sub>4</sub>	C <sub>18</sub> H <sub>14</sub> FN <sub>5</sub> OS	367.40	87
4	5d	4-NO <sub>2</sub> C <sub>6</sub> H <sub>4</sub>	C <sub>18</sub> H <sub>12</sub> FN <sub>5</sub> O <sub>3</sub> S	397.38	94
5	5e	2-NH <sub>2</sub> C <sub>6</sub> H <sub>4</sub>	C <sub>18</sub> H <sub>14</sub> FN <sub>5</sub> OS	367.40	82
6	5f	2-Cl C <sub>6</sub> H <sub>4</sub>	C <sub>18</sub> H <sub>12</sub> ClFN <sub>4</sub> OS	386.83	81
7	5g	4-Cl C <sub>6</sub> H <sub>4</sub>	C <sub>18</sub> H <sub>12</sub> ClFN <sub>4</sub> OS	386.83	74

8	5h	2-CH <sub>3</sub> C <sub>6</sub> H <sub>4</sub>	C <sub>19</sub> H <sub>15</sub> FN <sub>4</sub> OS	366.41	72
9	5i	3-NH <sub>2</sub> C <sub>6</sub> H <sub>4</sub>	C <sub>18</sub> H <sub>14</sub> FN <sub>5</sub> OS	367.40	86
10	5j	4-OMeC <sub>6</sub> H <sub>4</sub>	C <sub>19</sub> H <sub>15</sub> FN <sub>4</sub> O <sub>2</sub> S	382.41	83

## 2. 2. Analytical data

**6-(3-Chlorophenyl)-3-(6-fluorochroman-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (5a).** mp 150-154 °C; IR (DRS): 3073, 3031, 2957, 2847, 1625, 1462, 1442, 1325, 1258, 1140, 1065, 1018, 825, 748, 701, 685 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ ppm 2.44-2.48 (m, 1H, 2CH), 2.74-2.76 (m, 1H, 2CH), 3.04 (m, 2H, 2CH), 5.66-5.68 (m, 1H, CH), 6.82-6.84(m, 3H, ArH), 7.48-7.50(d, *J* = 5.79 Hz, 1H, ArH), 7.56(m, 1H, ArH), 7.73-7.75(d, *J* = 6.69 Hz, 1H, ArH), 7.90 (s, 1H, ArH). MS: *m/z* = 386 [M]<sup>+</sup>; Anal. Calcd for C<sub>18</sub>H<sub>12</sub>ClFN<sub>4</sub>OS: C, 55.89; H, 3.13; N, 14.48. Found: C, 55.83; H, 3.04; N, 14.08 %.

**6-(3,4-Dimethoxyphenyl)-3-(6-fluorochroman-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (5b).** mp 119-121 °C; IR (DRS): 3090, 3020, 2935, 2839, 1637, 1492, 1440, 1363, 1138, 1058, 1020, 810, 756, 705, 680 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, DMSO): δ ppm 2.41-2.44 (m, 1H, 2CH), 2.58-2.65 (m, 1H, 2CH), 3.02-3.19 (m, 2H, 2CH), 3.90 (s, 6H, OCH<sub>3</sub>, OCH<sub>3</sub>), 5.70-5.73 (d, d, *J* = 4.4 Hz, 3.4 Hz, 1H, CH), 6.78-6.93 (m, 3H, ArH), 7.09-7.11 (d, *J* = 8.44 Hz, 1H, ArH), 7.39 (s, 1H, ArH), 7.49-7.51 (d, *J* = 7.72 Hz, 1H, ArH). MS: *m/z* = 412 [M]<sup>+</sup>; Anal. Calcd for C<sub>20</sub>H<sub>17</sub>FN<sub>4</sub>O<sub>3</sub>S: C, 58.24; H, 4.15; N, 13.58. Found: C, 58.18; H, 3.99; N, 13.49 %.

**4-(3-(6-Fluorochroman-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl)aniline (5c).** mp 168-170 °C; IR (DRS): 3422, 3378, 3030, 2964, 2853, 1642, 1612, 1581, 1471, 1368, 1247, 1156, 1057, 1014, 819, 744, 710, 678 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, DMSO): δ ppm 7.74-7.17 (m, 2H), 6.76-6.62 (m, 4H), 6.50-6.48(dd, 1H), 5.24-5.20 (s, 2H), 5.16-5.13 (t, 1H), 3.17-3.15 (m, 2H), 2.56-2.54 (m, 1H), 2.36-2.34(dq, 1H). MS: *m/z* = 367 [M]<sup>+</sup>; Anal. Calcd for C<sub>18</sub>H<sub>14</sub>FN<sub>5</sub>OS: C, 58.84; H, 3.84; N, 19.06. Found: C, 58.69; H, 3.78; N, 18.90 %.

**3-(6-Fluorochroman-2-yl)-6-(4-nitrophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (5d).** mp 158-160 °C; IR (DRS): 3074, 2987, 2851, 1645, 1612, 1585, 1468, 1345, 1184, 1250, 1061, 1023, 820, 780, 744, 695, 566 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, DMSO): δ ppm 8.42-8.40 (m, 2H), 8.07-8.05 (m, 2H), 6.76-6.75 (m, 2H), 6.50-6.49 (dd, 1H), 5.15-5.13 (t, 1H), 3.17-3.15 (m, 2H), 2.52-2.50 (dq, 1H), 2.36-2.34 (dq, 1H). MS: *m/z* = 397 [M]<sup>+</sup>; Anal. Calcd for C<sub>18</sub>H<sub>12</sub>FN<sub>5</sub>O<sub>3</sub>S: C, 54.40; H, 3.04; N, 17.62. Found: C, 54.28; H, 2.93; N, 17.44 %.

**2-(3-(6-Fluorochroman-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl)aniline (5e).** mp 147-149 °C; IR (DRS): 3442, 3091, 3081, 2975, 2844, 1641, 1579, 1556, 1464, 1357, 1242, 1145, 1088, 1017, 832, 750, 687 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, DMSO): δ ppm 7.75-7.74 (dd, 1H), 7.22-7.20 (td, 1H), 7.01-7.00 (m, 2H), 6.75-6.73 (m, 2H), 6.50-6.49 (dd, 1H), 5.59-5.57 (s, 2H), 5.26-5.24 (t, 1H), 3.17-3.15 (m, 2H), 2.61-2.60 (dq, 1H), 2.34-2.32 (dq, 1H). MS: *m/z* =

367 [M]<sup>+</sup>; Anal. Calcd for C<sub>18</sub>H<sub>14</sub>FN<sub>5</sub>OS: C, 58.84; H, 3.84; N, 19.06. Found: C, 58.41; H, 3.78; N, 18.99 %.

**6-(4-Chlorophenyl)-3-(6-fluorochroman-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (5f).** mp 116-118 °C; IR (DRS): 3080, 2983, 2867, 1629, 1572, 1525, 1462, 1381, 1245, 1196, 1046, 1011, 830, 778, 701, 665, 578 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, DMSO): δ ppm 7.68-7.65 (m, 2H), 7.44-7.43 (m, 2H), 6.74-6.72 (m, 2H), 6.49-6.48 (dd, 1H), 5.26-5.24 (t, 1H), 3.17-3.16 (m, 2H), 2.60-2.58 (dt, 1H), 2.35-2.34 (dq, 1H) MS: *m/z* = 386 [M]<sup>+</sup>; Anal. Calcd for C<sub>18</sub>H<sub>12</sub>ClFN<sub>4</sub>OS: C, 55.89; H, 3.13; N, 14.48. Found: C, 55.84; H, 2.97; N, 14.17 %.

**6-(2-Chlorophenyl)-3-(6-fluorochroman-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (5g).** mp 183-185 °C; IR (DRS): 3077, 2978, 2863, 1625, 1609, 1563, 1464, 1331, 1238, 1142, 1038, 1014, 870, 832, 778, 668, 514 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, DMSO): δ ppm 8.00-7.98 (m, 2H), 7.65-7.63 (m, 2H), 6.76-6.74 (m, 2H), 6.50-6.48 (dd, 1H), 5.16-5.14 (t, 1H), 3.17-3.15 (m, 2H), 2.58-2.56 (m, 1H), 2.36-2.34 (dq, 1H). MS: *m/z* = 386 [M]<sup>+</sup>; Anal. Calcd for C<sub>18</sub>H<sub>12</sub>ClFN<sub>4</sub>OS: C, 55.89; H, 3.13; N, 14.48. Found: C, 55.67; H, 3.01; N, 14.21 %.

**3-(6-Fluorochroman-2-yl)-6-(*o*-tolyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6h).** mp 160-162 °C; IR (DRS): 3063, 2962, 2854, 1603, 1545, 1542, 1452, 1325, 1260, 1146, 1060, 1023, 812, 754, 662, 518 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, DMSO): δ ppm 7.80-7.79 (m, 1H), 7.40-7.38 (m, 4H), 6.75-6.73 (m, 3H), 6.50-6.48 (dd, 1H), 5.26-5.24 (t, 1H), 3.17-1.15 (s, 3H), 2.61-2.60 (dq, 1H), 2.34-2.33 (dq, 1H). MS: *m/z* = 366 [M]<sup>+</sup>; Anal. Calcd for C<sub>19</sub>H<sub>15</sub>FN<sub>4</sub>OS: C, 62.28; H, 4.13; N, 15.29. Found: C, 62.19; H, 3.97; N, 15.24 %.

**3-(3-(6-Fluorochroman-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl)aniline (5i).** mp 109-111 °C; IR (DRS): 3428, 3392, 3075, 2964, 2853, 1721, 1601, 1581, 1423, 1371, 1241, 1149, 1054, 1026, 888, 848, 766, 720, 665, 578 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, DMSO): δ ppm 7.71-7.59 (m, 2H), 7.27 (t, 1H), 6.83 (dt, 1H), 6.76-6.66 (m, 2H), 5.19 (t, 1H), 4.26 (s, 2H), 3.20-3.03 (m, 2H), 2.51 (dq, 1H), 2.35 (dq, 1H). MS: *m/z* = 367 [M]<sup>+</sup>; Anal. Calcd for C<sub>18</sub>H<sub>14</sub>FN<sub>5</sub>OS: C, 58.84; H, 3.84; N, 19.06. Found: C, 58.53; H, 3.71; N, 18.90 %.

**3-(6-Fluorochroman-2-yl)-6-(4-methoxyphenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (5j).** mp 224-226 °C; IR (DRS): 3061, 2951, 2872, 1689, 1589, 1579, 1462, 1354, 1208, 1135, 1099, 1003, 819, 755, 688 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, DMSO): δ ppm 7.82 (dt, 1H), 7.65 (t, 1H), 7.38 (t, 1H), 6.89 (dt, 1H), 6.76-6.65 (m, 2H), 6.44 (dd, 1H), 5.15 (t, 1H), 3.79 (s, 3H), 3.17 (m, 2H), 2.52 (dq, 1H), 2.36 (dq, 1H). MS: *m/z* = 382 [M]<sup>+</sup>; Anal. Calcd for C<sub>19</sub>H<sub>15</sub>FN<sub>4</sub>O<sub>2</sub>S: C, 59.67; H, 3.95; N, 14.65. Found: C, 59.08; H, 3.88; N, 14.62 %.

### 3. ANTI MICROBIAL ACTIVITY

All the glass apparatus used were sterilized before use. The broth dilution technique was used to determine the minimum inhibitory concentration (MIC) of the synthesized compounds. Bacterial strain of *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Escherichia coli* and fungal strains of *Aspergillus niger*, *Candida albicans* and *Aspergillus clavatus* were used in the present study. DMSO was used as the solvent for the compounds. A blank test was carried out to check the antimicrobial activity of DMSO.

Ampicillin and Chloramphenicol were used as the standard drugs for antibacterial activity. Greseofulvin was used as the standard drug for antifungal activity. The synthesized 1,2,4-triazolo-[3,4-b]thiadiazole 5a-5j were screened for their antimicrobial activity by the broth dilution method to evaluate the minimum inhibitory concentration Table 2.

**Table 2.** Antimicrobial activity of 1,2,4-triazolo-[3,4-b]thiadiazole derivatives (5a-5j).

Sr. No.	Antibacterial Activity				Antifungal activity		
	Minimal bactericidal concentration $\mu\text{g/ml}$				Minimal fungicidal concentration $\mu\text{g/ml}$		
	Gram +ve Bacteria		Gram -ve Bacteria		C. albicans	A. niger	A. clavatus
	S. aureus	S. pyogenus	E. coli	P. aeruginosa			
5a	100	62.5	250	250	1000	>1000	>1000
5b	250	250	250	200	>1000	>1000	>1000
5c	125	100	250	100	>1000	>1000	>1000
5d	200	200	100	200	1000	>1000	>1000
5e	62.5	125	62.5	100	500	1000	1000
5f	125	200	100	125	>1000	250	500
5g	500	500	250	200	500	1000	1000
5h	500	250	200	100	>1000	>1000	>1000
5i	500	500	100	100	1000	250	250
5j	250	500	250	250	500	500	500
<b>MINIMAL INHIBITION CONCENTRATION</b>							
Standard Drugs		S. aureus		S. pyogenus	E. coli	P. aeruginosa	
		(microgramme/ml)					
Ampicillin		250		100	100	100	
Chloramphenicol		50		50	50	50	
<b>MINIMAL FUNGICIDAL CONCENTRATION</b>							
Standard Drugs		C. Albicans		A. Niger	A. Clavatus		
		(microgramme/ml)					
Greseofulvin		500		100	100		

All of the precursors (5a-j) of the title compounds showed antibacterial activity in the range of 62.5-500  $\mu\text{g/mL}$  for *Staphylococcus aureus*, 100-500  $\mu\text{g/mL}$  for *Streptococcus pyogenes*, 100-500  $\mu\text{g/mL}$  for *Pseudomonas aeruginosa*, and 62.5-500  $\mu\text{g/mL}$  for *Escherichia coli*. It was observed that compound 5e (MIC = 62.5  $\mu\text{g/mL}$ ) against *s. aureus* as well as compound 5e (MIC = 62.5  $\mu\text{g/mL}$ ) against *E.coli* have found to be better active as

compared to ampicillin (MIC = 250 µg/mL). Except compound 5e, 5a and 5c compounds 5b, 5f, 5d, 5g and 5i were found moderately active against *S. aureus*, *p. aeruginosa* and *Escherichia coli* as compared to ampicillin. Against fungal pathogen *C. albicans* 5g, 5e and 5j have shown good activity as compared to griseofulvin (MIC = 500 µg/ml).

#### 4. CONCLUSION

In present report, we are reporting very efficient method for the synthesis of some novel 1,2,4-triazolo-[3,4-b]thiadiazole derivatives possessing 6-fluoro chroman nucleus. All synthesized compounds were obtained in good yield. From the results of antimicrobial data, compounds 5e showed excellent results against Gram positive and Gram negative bacteria while compounds 5a, 5c and 5f were found moderate active. All synthesized compounds showed minimal activity against fungi pathogens as compared to the standard drugs.

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