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Short communication

Synthesis of pharmaceutically important condensed heterocyclic 4,6-disubstituted-1,2,4-triazolo-1,3,4-thiadiazole derivatives as antimicrobials

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Abstract

The two series of 4,6-disubstituted 1,2,4-triazolo-1,3,4-thiadiazole derivatives **2(a–e)** and **3(a–e)** were synthesized and characterized using IR, ^{1}H-NMR, CHNS analysis and by single crystal X-ray crystallographic studies. The compound 6-(2-chloro-phenyl)-3-ethyl-[1,2,4]triazole[3,4-b] thiadiazole **2b** has been characterized by single crystal X-ray diffraction method. The compound crystallizes in monoclinic space group $P2_1/c$ with a cell parameters $a = 11.879(1)$ Å, $b = 15.112(2)$ Å, $c = 13.95(2)$ Å, $Z = 8$ and the final R factor is $R1 = 0.0524$. The structure exhibits both intra and intermolecular hydrogen bonds. The title compounds were checked for their efficacy as antimicrobials in vitro. Compounds **2b**, **2c, 2d, 3b, 3c** and **3d** showed significant inhibition against all the strains tested, when compared to standard drugs.

Keywords: 1,2,4-Triazolo-1,3,4-thiadiazole; Antimicrobials; Intra/inter-molecular hydrogen bonds; Crystal structure

1. Introduction

Antimicrobials reduce or completely block the growth and multiplication of bacteria. This has made them unique for the control of deadly infectious diseases caused by a variety of pathogens. They have transformed our ability to treat infectious diseases such as pneumonia, meningitis, tuberculosis, malaria and AIDS. Derivatives of 1,2,4-triazole and 1,3,4-thiadiazole condensed nucleus system found to have diverse pharmacological activities [1] such as fungicidal, insecticidal, bactericidal, herbicidal, anti-tumor [2], anti-inflammatory [3], CNS stimulant properties [4]. They also find applications as dyes, lubricants and analytical reagents [5], antiviral agents [6]. Examples of such compounds bearing the 1,2,4-triazole moieties are fluconazole, a powerful azole antifungal agent [7] as well as the potent antiviral N-nucleoside ribavirin [6]. Also, a number of 1,3,4-thiadiazoles showed antibacterial properties similar to those of well-known sulfonamide drugs [8]. The thiadiazole nucleus with N–C–S linkage exhibits a large number of biological activities [1]. Prompted by these findings and in continuation of our efforts in synthesizing various condensed bridge bioactive molecules, bearing multifunctional and pharmaceutically active groups [9–14], herein, we report the synthesis and antimicrobial activity in vitro of a series of 4,6-substituted 1,2,4-triazolo-1,3,4-thiadiazole derivatives.

2. Chemistry

Initially, the 4-amino-3-substituted aryl-5-mercapto-[1,2,4]-triazoles **1(c–e)** were synthesized by the conversion of the substituted aryl esters into their corresponding hydrazides by using hydrazine hydrate and the subsequent reactivity of the hydrazides with carbon disulphide in presence of alcoholic KOH to obtain the potassium salts of thiocarbohydrazides, followed by the addition of the hydrazine hydrate gives the cyclized com-

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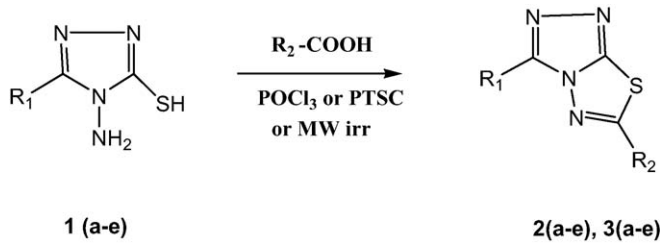
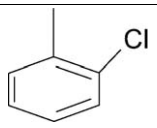
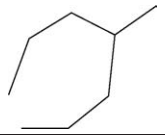


Scheme 1.

Table 1
Physical data of 1,2,4-triazolo-1,3,4-thiadiazoles

Compound	R_1	Compound	R_2	R_f value	M.p. °C
1a	$-CH_3$	**2a**		0.39	180
1b	$-CH_2-CH_3$	**2b**		0.41	110
1c	$-C_6H_5$	**2c**		0.44	135
1d	$-4-CH_3-C_6H_5$	**2d**		0.48	160
1e	$-4-Cl-C_6H_5$	**2e**		0.54	175
1a	$-CH_3$	**3a**		0.42	Oil
1b	$-CH_2-CH_3$	**3b**		0.45	Oil
1c	$-C_6H_5$	**3c**		0.48	65
1d	$-4-CH_3-C_6H_5$	**3d**		0.50	76
1e	$-4-Cl-C_6H_5$	**3e**		0.52	83

pounds, which involved the reported procedure [15]. Also, the condensation of thiocarbohydrazide with aliphatic acids gave 4-amino-3-alkyl-5-mercapto-[1,2,4]-triazoles **1(a–b)**. The title compounds **2(a–e)** and **3(a–e)** were synthesized by using simple, efficient and one-pot condensation of 4-amino-3-alkyl or aryl substituted-5-mercapto-1,2,4-triazoles **1(a–e)** with 2-chloro benzoic acid and valproic acid under different conditions including microwave irradiation in DMF solvent [12].

Condensation of **1(a–e)** with the acids either in $POCl_3$ at reflux temperature for overnight or with para-toluene sulfonyl chloride in toluene for 12 hours at 65–70 °C or microwave irradiation (Kenstar) in DMF as a solvent for about 30–40 s at 60 power unit produced **2(a–e)** and **3(a–e),** with 65–68%, 70–75% and 80–82% yield, respectively (Scheme 1 and Table 1).

3. Results and discussion

3.1. Chemistry

The condensation reaction to yield the title compounds was found to be selective by microwave irradiation technique with good yield in short time. The products were separated on silica gel column using appropriate combination of n-hexane and ethyl acetate as eluent. All the compounds were structurally characterized by using IR, ^{1}H-NMR and CHNS analysis. The IR absorption band around 1285 cm^{-1} could be attributed to the –C–S– functional group. The substituted 1,2,4-triazole derivatives showed the peak around 13.8 represents the thiol group, which clearly disappeared in the title compounds confirms their condensed structure.

3.2. Crystal structure analysis of *2b*

Previously, we have reported the single crystal X-ray crystallographic studies of the compound 6-(2-chlorophenyl)-3-methyl-[1,2,4]-triazolo[4,5-b][1,3,4]thiadiazole 2a, in which the condensed nucleus is coplanar with the phenyl ring [12]. In the present study, we herein, report the single crystal X-ray crystallographic studies of 6-(2-chloro-phenyl)-3-ethyl-[1,2,4]triazole [3,4-b]thiadiazole **2b** to know the condensed nucleus structural orientation. Good quality single crystal was obtained from the slow evaporation technique using ethyl acetate as solvent. Crystal and the experimental data are shown in Table 2. The selected bond distances and angles of non-hydrogen atoms, respectively, are as shown in Table 3 and hydrogen atoms were placed at chemically acceptable positions. The bond distances and bond angles are in good agreement with the standard values. There are two molecules in an asymmetric unit. Fig. 1 represents the ORTEP diagram of the molecule with thermal ellipsoids at 50% probability [16]. The packing of the molecules shows layered stacking when viewed down *b* axis. The five-membered and phenyl rings of both molecules are planar with the maximum deviation of 0.021(5) Å for C12A. The structure exhibits intermolecular hydrogen bonds of the type C–H…N. The intra and intermolecular hydrogen bonds are: C17B–H1B2…N13B (3.525(8) Å, 163°), C17A–H1A2…N13A (3.544(9) Å, 165°), C4B–H13B…N13B (3.443(7) Å, 149°), C3A–H14A…N14A (3.269(8) Å, 131°) and C3B–H14B…N14B (3.233(7) Å, 130° with symmetry codes ($-x$, $1/2 + y$, $1/2 - z$), ($1 - x$, $-1/2 + y$, $3/2 - z$), ($1 + x$, $3/2 - y$, $1/2 + z$), ($-1 + x$, $1/2 - y$, $-1/2 + z$) and ($1 + x$, $3/2 - y$, $1/2 + z$), respectively.

Table 2
Crystal data and structure refinement of **2b**

Empirical formula	$C_{11}H_9ClN_4OS$
Formula weight	264.73
Temperature	293(2) K
Wavelength	0.71069 Å
Crystal system	Monoclinic
Space group	*P*21/*c*
Cell dimensions	
$a = 11.879(14)$ Å, $b = 15.112(19)$, Å, $c = 13.95(2)$ Å	
$\alpha = 90°$, $\beta = 112.69\ (9)°$, $\gamma = 90°$	
Z	8
Volume	2310 (5) $Å^3$
Density (calculated)	1.523 mg m^{-3}
Absorption Coefficient	0.492 mm^{-1}
*F*000	1088
Crystal size	0.2 × 0.2 × 0.2 mm
Theta range for data collection	2.08° to 35.52°
Index ranges	$0 \le h \le 17$
	$0 \le k \le 22$
	$-21 \le 1 \le 19$
Reflections collected	8660
Independent reflections	8283 [*R* (int) = 0.0270]
Refinement method	Full-matrix least-squares o
Data/restrains/parameters	8283/0/309
Goodness-of-fit on F^2	0.978
Final *R* indices [$I > 2\sigma(I)$]	$R_1 = 0.0524$, $\omega R_2 = 0.1526$
	$R_1 = 0.1708$, $\omega R_2 = 0.2271$
Largest diff. peak and hole	0.471 and –0.825 *e* A^{-3}

Table 3
Selected bond lengths (Å) and angles (°)

Atoms	Length	Atoms	Length
S8B–C12B	1.722(4)	S8A–C12A	1.748(5)
S8B–C9B	1.786(5)	S8A–C9A	1.755(5)
Cl1B–C2B	1.722(6)	Cl1A–C2A	1.742(5)
N11B–C12B	1.338(6)	N11A–C15A	1.344(5)
N11B–N10B	1.339(4)	N11A–C12A	1.381(6)
N11B–C15B	1.389(5)	N11A–N10A	1.397(4)
N10B–C9B	1.336(5)	N10A–C9A	1.269(6)
N13B–C12B	1.355(5)	N13A–C12A	1.253(5)
N13B–N14B	1.424(5)	N13A–N14A	1.386(6)
C15B–N14B	1.332(5)	N14A–C15A	1.304(5)
C9B–C7B	1.422(5)	C9A–C7A	1.526(5)
Atoms	Angle	Atoms	Angle
C12B–S8B–C9B	88.1(2)	C12A–S8A–C9A	88.0(2)
C12B–N11B–N10B	121.0(3)	C15A–N11A–C12A	106.4(4)
C12B–N11B–C15B	105.9(3)	C15A–N11A–N10A	136.9(4)
N10B–N11B–C15B	133.1(4)	C12A–N11A–N10A	116.7(4)
C9B–N10B–N11B	107.8(3)	C9A–N10A–N11A	109.1(4)
C12B–N13B–N14B	103.5(3)	C12A–N13A–N14A	106.0(4)
C3B–C2B–Cl1B	116.2(3)	C7A–C2A–Cl1A	122.9(3)
C7B–C2B–Cl1B	121.7(4)	C3A–C2A–Cl1A	116.6(4)
N11B–C12B–N13B	112.8(4)	N13A–C12A–N11A	110.7(4)
N11B–C12B–S8B	109.0(3)	N13A–C12A–S8A	140.6(4)
N13B–C12B–S8B	138.2(4)	N11A–C12A–S8A	108.6(3)
N14B–C15B–N11B	108.7(4)	C15A–N14A–N13A	110.2(4)
N14B–C15B–C16B	125.3(4)	N10A–C9A–C7A	118.4(4)
N11B–C15B–C16B	126.0(4)	N10A–C9A–S8A	117.6(3)
N10B-C9B-C7B	119.3(4)	C7A–C9A–S8A	124.0(3)
N10B–C9B–S8B	114.1(3)	N14A–C15A–N11A	106.6(4)
C7B–C9B–S8B	126.5(3)	N14A–C15A–C16A	128.8(4)
C15B–N14B–N13B	109.0(3)	N11A–C15A–C16A	124.5(4)

3.3. Antimicrobial activity

In an approach to develop new antimicrobial agents, we have synthesized 4,6-disubstituted 1,2,4-triazolo-1,3,4-thiadiazole derivatives **2**(**a–e**) and **3**(**a–e**) and evaluated for their efficacy as antimicrobials in vitro by disk diffusion method against the various pathogenic strains. Nystatin was used as standard drug against fungi, streptomycin and tetracycline against bacteria. In all the determinations, tests are performed in triplicate and the results were reported as mean of at least three determinations. Our results showed that the compounds, **2b, 2c**, **2d, 3b**, **3c** and **3d** were effective compared to standard drugs against the bacterial and fungal strains tested as shown in Tables 4–7. The structures of the compounds that showed significant activity are shown in Fig. 2. Compounds **2b**, **2c** and **2d** bearing ethyl, phenyl and *p*-tolyl groups exhibited significant inhibitory activity, this might be possibly due to the presence of electron releasing ability by either ethyl, phenyl or *p*-tolyl groups to the condensed nucleus. Compound **2e** did not show any potency, might be due to the electron-withdrawing effect of the chlorine atom. Second series of compounds **3**(**a–e**) are the derivatives of valproic acid. Valproic acid is one of the potent inhibitors of histone deacetylase, an enzyme mainly involved in the transcription mediated diseases like cancer. We found that the compounds **3b, 3c** and **3d** are potent antimicrobials; this is possibly due to the electron inductive effect by both the substituents (alkyl chain) at the 6th and 3rd position (Fig. 2).

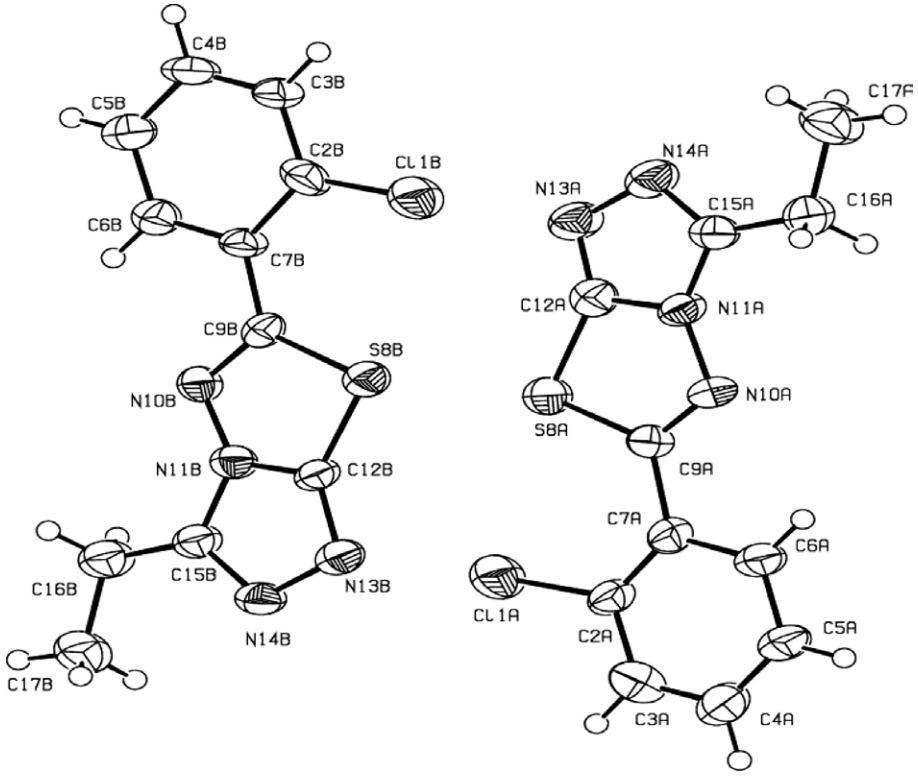


Fig. 1. Oak Ridge Thermal Ellipsoid Plot of the molecule **2b** at 50% probability.

Table 4
Inhibitory Zone (diameter) mm of synthesized compounds against tested bacterial strains by disk diffusion method

Compounds	Inhibitory zone (diameter; mm)[a]				
	Bacillus subtilis	*Escherichia coli*	*Pseudomonas fluorescens*	*Xanthomonas campestris pvs*	*Xanthomonas oryzae*
2a	3 ± 0.11	4 ± 0.16	1 ± 0.03	2 ± 0.08	5 ± 0.21
2b	16 ± 0.71	15 ± 0.7	17 ± 0.71	14 ± 0.6	15 ± 0.58
2c	17 ± 0.74	19 ± 0.82	20 ± 0.92	16 ± 0.7	17 ± 0.69
2d	19 ± 0.82	21 ± 0.98	22 ± 0.95	19 ± 0.89	18 ± 0.79
2e	4 ± 0.18	5 ± 0.2	6 ± 0.21	3 ± 0.12	2 ± 0.09
3a	5 ± 0.19	6 ± 0.22	3 ± 0.1	4 ± 0.19	1 ± 0.02
3b	17 ± 0.75	18 ± 0.79	20 ± 0.94	15 ± 0.71	14 ± 0.59
3c	19 ± 0.85	20 ± 0.96	23 ± 1.1	18 ± 0.81	17 ± 0.69
3d	20 ± 0.94	22 ± 0.98	24 ± 1.14	21 ± 1.0	20 ± 0.89
3e	4 ± 0.17	5 ± 0.17	2 ± 0.07	6 ± 0.24	3 ± 0.12
Streptomycin	12 ± 0.51	14 ± 0.61	18 ± 0.78	–	–
Tetracycline	–	–	–	12 ± 0.49	11 ± 0.52
DMSO	0.8 ± 0.03	0.6 ± 0.01	0.9 ± 0.031	0.5 ± 0.02	0.7 ± 0.025

Streptomycin sulfate (10 μg per disc); tetracycline (10 μg per disc) were used as standard antibiotic discs, synthesized compounds (25 μg per disc).
[a] Values are mean of three determinations, the ranges of which are less than 5% of the mean in all cases.

Table 5
Inhibitory zone (diameter) mm of synthesized compounds against tested fungal strains by disk diffusion method

Compounds	Inhibitory zone (diameter; mm)[a]				
	Aspergillus niger	*Aspergillus flavus*	*Fusarium oxysporum*	*Trichoderma sp*	*Fusarium monaliforme*
2a	3 ± 0.11	4 ± 0.15	2 ± 0.7	5 ± 0.19	1 ± 0.03
2b	17 ± 0.75	14 ± 0.6	18 ± 0.6	19 ± 0.9	13 ± 0.6
2c	19 ± 0.90	16 ± 0.6	20 ± 0.8	21 ± 1.1	15 ± 0.8
2d	21 ± 1	18 ± 0.8	22 ± 0.9	24 ± 0.9	17 ± 0.9
2e	4 ± 0.14	6 ± 0.25	3 ± 0.9	6 ± 0.21	5 ± 0.18
3a	5 ± 0.2	4 ± 0.17	3 ± 0.8	4 ± 0.15	2 ± 0.6
3b	16 ± 0.7	15 ± 0.7	19 ± 0.8	21 ± 1	18 ± 0.9
3c	18 ± 0.8	17 ± 0.8	21 ± 0.95	23 ± 1.1	19 ± 0.82
3d	22 ± 0.9	20 ± 0.9	24 ± 1	26 ± 1.1	21 ± 0.91
3e	4 ± 0.15	3 ± 0.11	2 ± 0.08	5 ± 0.2	1 ± 0.03
Nystatin	8 ± 0.3	10 ± 0.3	14 ± 0.55	16 ± 0.6	12 ± 0.45
DMSO	0.8 ± 0.03	0.6 ± 0.02	0.5 ± 0.021	0.3 ± 0.012	0.9 ± 0.03

Nystatin (10 μg per disc) was used as standard antibiotic disc, synthesized compounds (25 μg per disc).
[a] Values are mean of three determinations, the ranges of which are less than 5% of the mean in all cases.

Table 6
Minimal inhibitory concentration (MIC) in μg ml^{-1} of compounds against tested bacterial strains by microdilution method

Compounds	Minimal inhibitory concentration (MIC) in μg ml^{-1}[a]				
	Bacillus subtilis	*Escherichia coli*	*Pseudomonas fluorescens*	*Xanthomonas campestris pvs*	*Xanthomonas oryzae*
2a	38 ± 1.7	41 ± 1.8	44 ± 2	39 ± 1.7	42 ± 1.7
2b	11 ± 0.42	13 ± 0.48	14 ± 0.6	12 ± 0.42	10 ± 0.4
2c	13 ± 0.5	15 ± 0.6	16 ± 0.7	13 ± 0.5	14 ± 0.5
2d	14 ± 0.6	17 ± 0.7	18 ± 0.75	15 ± 0.62	16 ± 0.52
2e	36 ± 0.15	40 ± 1.5	42 ± 1.7	35 ± 1.6	37 ± 1.6
3a	37 ± 1.5	39 ± 1.4	42 ± 1.5	37 ± 1.5	40 ± 1.6
3b	10 ± 0.41	12 ± 0.41	11 ± 0.4	9 ± 0.4	8 ± 0.3
3c	12 ± 0.5	14 ± 0.52	15 ± 0.7	14 ± 0.52	13 ± 0.5
3d	13 ± 0.42	15 ± 0.6	16 ± 0.65	15 ± 0.6	16 ± 0.7
3e	35 ± 1.3	38 ± 1.6	40 ± 1.6	34 ± 1.4	36 ± 1.4
Streptomycin	25 ± 1.2	19 ± 0.78	17 ± 0.7	–	–
Tetracycline	–	–	–	13 ± 0.5	19 ± 0.8
DMSO	1 ± 0.03	0.9 ± 0.02	0.7 ± 0.02	0.8 ± 0.03	0.5 ± 0.02

[a] Values are mean of three determinations, the ranges of which are less than 5% of the mean in all cases.

4. Conclusion

In conclusion, we report the antimicrobial studies of newly synthesized 4,6-disubstituted 1,2,4-triazolo-1, 3,4-thiadiazole derivatives. Compounds **3d**, **3c**, **3b**, **2d**, **2c** and **2b** showed potent inhibition against all the bacterial and fungal strains tested and found to be non-strain dependent. Further, the research on modification of the title compounds to understand the structure activity relationship and the mechanism of inhibition is underway.

Table 7
Minimal inhibitory concentration (MIC) in μM of compounds against tested fungal strains by turbidometric method

Compounds	Minimal inhibitory concentration (MIC) in μM[a]				
	Aspergillus niger	*Aspergillus flavus*	*Fusarium oxysporum*	*Trichoderma* sp	*Fusarium monaliforme*
2a	30 ± 1.2	24 ± 1	26 ± 1.1	22 ± 0.9	27 ± 1
2b	11 ± 0.43	10 ± 0.35	9 ± 0.38	8 ± 0.3	12 ± 0.42
2c	13 ± 0.42	12 ± 0.45	14 ± 0.52	10 ± 0.4	13 ± 0.5
2d	16 ± 0.5	14 ± 0.6	15 ± 0.5	11 ± 0.45	16 ± 0.6
2e	32 ± 1.4	29 ± ±1.2	33 ± 1.4	30 ± 1.1	34 ± 1.5
3a	28 ± 1.2	22 ± 0.9	24 ± 1	20 ± 0.9	25 ± 1.1
3b	11 ± 0.5	9 ± 0.4	8 ± 0.3	7 ± 0.3	10 ± 0.41
3c	13 ± 0.46	12 ± 0.5	13 ± 0.5	10 ± 0.4	15 ± 0.55
3d	14 ± 0.6	13 ± 0.48	15 ± 0.51	11 ± 0.42	16 ± 0.6
3e	30 ± 1.2	26 ± 1.1	28 ± 1.1	24 ± 1	30 ± 1.2
Nystatin	29 ± 1.2	34 ± 1.5	36 ± 1.6	30 ± 1.2	32 ± 1.2
DMSO	0.4 ± 0.1	0.6 ± 0.2	0.9 ± 0.3	0.8 ± 0.3	0.7 ± 0.2

[a] Values are means of three determinations, the ranges of which are less than 5% of the mean in all cases.

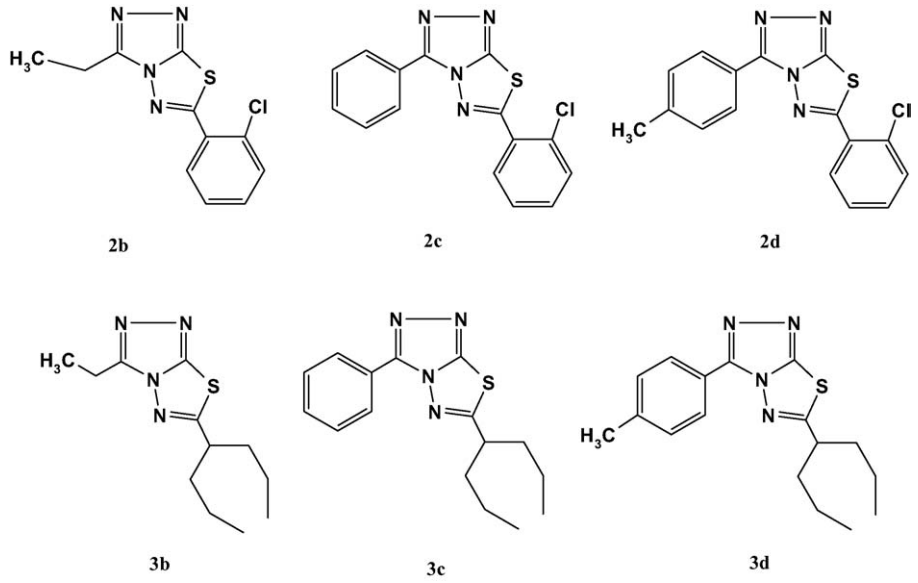


Fig. 2. Structures of the potent molecules.

5. Experimental

5.1. Chemistry

The melting points were determined on SELACO-650 hot stage apparatus and are uncorrected. IR (KBr) spectra were recorded on a Jasco FT/IR-4100 Fourier transform infrared spectrometer, ^{1}H-NMR were recorded on Shimadzu AMX 400, spectrometer by using $CDCl_3$ as solvent and TMS as an internal standard (Chemical shift in δ ppm). Elemental analyses were obtained on a Vario-EL instrument. Thin layer chromatography (TLC) was conducted on 0.25 mm silica gel plates ($60F_{254}$, Merck). Visualization was made with ultraviolet light. All extracted solvents were dried over Na_2SO_4 and evaporated with a BUCHI rotary evaporator. Reagents were obtained commercially and used as received.

5.1.1. Synthesis of 6-(2-chloro-phenyl)-3-methyl-[1,2,4] triazolo[3,4-b][1,3,4]thiadiazole **2a**

It was obtained from **1a** (0.5 g, 3.846 mmol), 2-chlorobenzoic acid (0.66 g, 4.230 mmol) [12].

5.1.2. Synthesis of 6-(2-chloro-phenyl)-3-ethyl-[1,2,4]triazolo [3,4-b][1,3,4]thiadiazole **2b**

It was obtained from **1b** (0.5 g, 3.472 mmol), 2-chlorobenzoic acid (0.595 g, 3.819 mmol). The product obtained was pale yellow solid (0.45 g, 90%).

IR (cm $^{-1}$ Nujol): 3120, 1225, 1530, 732.

^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.31–1.90 (t, 3H, Ar–H), 3.01–3.11 (q, 2H, $–CH_3$), 7.58–7.67 (t, 1H, Ar–H), 7.69–7.70 (t, 1H, Ar–H), 7.74–7.76 (d, 1H, $J = 8$ Hz, Ar–H), 8.01–8.03 (d, 1H, $J = 7$ Hz, Ar–H).

Anal. Calcd for $C_{11}H_9ClN_4S$: C, 49.91; H, 3.43; N, 21.16; S, 12.11 **Found**: C, 49.87; H, 3.28; N, 21.25; S, 12.01.

*5.1.3. Synthesis of 6-(2-chloro-phenyl)-3-phenyl-[1,2,4] triazolo[3,4-b] [1,3,4]thiadiazole **2c***

It was obtained from **1c** (0.5 g, 2.6041 mmol), 2-chlorobenzoic acid (0.446 g, 2.864 mmol). The product obtained was pale yellow solid (0.449 g, 88%) [24].

IR (cm -1 Nujol): 3107, 1230, 1550, 734.

^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.56–7.66 (m, 5H, Ar–H), 7.69–7.79 (t, 1H, Ar–H), 7.77–7.80 (dd, 1H, $J = 2$ Hz, $J = 2$ Hz, Ar–H), 8.11–8.19 (dd, 1H, $J = 1$ Hz, $J = 1$ Hz, Ar–H), 8.29–8.31 (d, 1H, $J = 8$ Hz, Ar–H).

*5.1.4. Synthesis of 6-(2-chloro-phenyl)-3-p-tolyl-[1,2,4]triazolo [3,4-b][1,3,4]thiadiazole **2d***

It was obtained from **1d** (0.5 g, 2.427 m mol), 2-chlorobenzoic acid (0.416 g, 2.669 m mol. The product obtained was yellow solid (0.375 g, 75%).

IR (cm $^{-1}$ Nujol): 3115, 1235, 1565, 725.

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.56–7.66 (m, 5H, Ar–H), 7.69–7.79 (t, 1H, Ar–H), 7.77–7.80 (dd, 1H, $J = 2$ Hz, $J = 2$ Hz, Ar–H), 8.11–8.26 (dd, 1H, $J = 1$ Hz, $J = 1$ Hz, Ar–H), 8.29–8.31 (d, 1H, $J = 8$ Hz, Ar–H).

Anal. Calcd for $C_{16}H_{11}ClN_4S$: C, 58.80; H, 3.39; N, 17.14; S, 9.81 **Found**: C, 58.78; H, 3.24; N, 17.28; S, 9.76.

*5.1.5. Synthesis of 6(2-chlorophenyl)-3-(4-chlorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole **2e***

It was obtained from **1e** (0.5 g, 2.212 mmol), 2-chlorobenzoic acid (0.3796 g, 2.433 mmol). The product obtained was pale yellow solid (0.39 g, 79%).

IR (cm $^{-1}$ Nujol): 3125, 1240, 1580, 729.

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.62–7.66 (t, 1H, Ar–H), 7.70–7.74 (m, 3H, Ar–H), 7.74–7.78 (d, 1H, $J = 16$ Hz, Ar–H), 8.13–8.21 (dd, 1H, $J = 1$ Hz, $J = 2$ Hz, Ar–H), 8.30–8.32 (d, 2H, $J = 9$ Hz, Ar–H).

Anal. Calcd for $C_{15}H_8Cl_2N_4S$: C, 51.89; H, 2.32; N, 16.14; S, 9.23 **Found**: C, 51.75; H, 2.42; N, 16.05; S, 9.46.

*5.1.6. Synthesis of 3-methyl-6-(1-propyl-butyl)-[1,2,4]triazolo [3,4-b][1,3,4]thiadiazole **3a***

It was obtained from **1a** (0.5 g, 3.846 mmol), Valproic acid (0.61 g, 4.23 mmol). The product obtained was oil (0.38 g, 76%).

IR (cm $^{-1}$ Nujol): 3030, 1625, 1249, 687.

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.71–0.80 (t, 6H, $–CH_3$), 1.10–1.26 (m, 4H, $–CH_2–CH_3$), 1.47–1.62 (m, 4H, $–CH–CH_2–$), 2.95–3.07 (s, 3H, $Tr–CH_3$), 3.97–4.08 (quintet, 1H, Ar–H).

Anal. Calcd. for $C_{11}H_{18}N_4S$: C, 55.43; H, 7.61; N, 23.51; S, 13.45 **Found**: C, 55.38; H, 7.46; N, 23.48; S, 13.32.

*5.1.7. Synthesis of 3-ethyl-6-(1-propyl-butyl)-[1,2,4]triazolo [3,4-b][1,3,4]thiadiazole **3b***

It was obtained from **1b** (0.5 g, 3.472 mmol), Valproic acid (0.55 g, 3.8 mmol). The product obtained was yellow oil (0.41 g, 82%).

IR (cm $^{-1}$ Nujol): 3131, 1624, 1240, 691.

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.71–0.80 (t, 6H, $–CH_3$), 1.20–1.38 (m, 4H, $–CH_2–CH_3$), 1.60–1.76 (m, 4H, $–CH–CH_2$), 2.46–2.52 (t, 3H, $Tr–CH_3$), 2.92–3.05 (q, 2H, $–CH_2–Tr$), 4.10–4.20 (quintet, 1H, Ar–H).

Anal. Calcd. for $C_{12}H_{20}N_4S$: C, 57.11; H, 7.99; N, 22.20; S, 12.71 **Found**: C, 57.05; H, 7.79; N, 22.18; S, 12.86.

*5.1.8. Synthesis of 3-phenyl-6-(1-propyl-butyl)-[1,2,4]triazolo [3,4-b][1,3,4]thiadiazole **3c***

It was obtained from **1c** (0.5 g, 2.604 mmol), Valproic acid (0.41 g, 2.86 mmol). The product obtained was pale yellow solid (0.405 g, 91%).

IR (cm $^{-1}$ Nujol): 3111, 1255, 1622, 699 (C-S-C).

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.82–0.92 (t, 6H, $–CH_3$), 1.19–1.40 (m, 4H, $–CH_2–CH_3$), 1.60–1.78 (m, 4H, $–CH–CH_2$), 3.98–4.18 (quintet, 1H, Ar–H), 7.50–7.76 (m, 3H, Ar–H), 8.18–8.27 (d, 2H, $J = 8$ Hz, Ar–H).

Anal. Calcd. for $C_{16}H_{20}N_4S$: C, 63.97; H, 6.71; N, 18.65; S, 10.67 **Found**: C, 63.86; H, 6.65; N, 18.45; S, 10.74.

*5.1.9. Synthesis of 6-(1-propyl-butyl)-3-p-tolyl-[1,2,4]triazolo [3,4-b][1,3,4]thiadiazole **3d***

It was obtained from **1d** (0.5 g, 2.4272 mmol), Valproic acid (0.385 g, 2.66 mmol). The product obtained was pale yellow solid (0.425 g, 85%).

IR (cm $^{-1}$ Nujol): 3063, 1626, 1262, 696.

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.81–0.95 (t, 6H, $–CH_3$), 1.22–1.40 (m, 4H, $–CH_2–CH_3$), 1.60–1.82 (m, 4H, $–CH–CH_2$), 4.10–4.17 (quintet, 1H, Ar–H), 7.53–7.76 (dd, 3H, $J = 2$ Hz, $J = 5$ Hz, Ar–H), 8.20–8.30 (dd, 2H, $J = 8$ Hz, $J = 14$ Hz, Ar–H).

Anal. Calcd. for $C_{17}H_{22}N_4S$: C, 64.93; H, 7.05; N, 17.82; S, 10.20 Found: C, 64.84; H, 7.14; N, 17.79; S, 10.16.

*5.1.10. Synthesis of 3-(4-chloro-phenyl)-6-(1-propyl-butyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole **3e***

It was obtained from 1e (0.5 g, 2.2208 mmol), Valproic acid (0.352 g, 2.44 mmol). The product obtained was pale yellow solid (0.40 g, 80%).

IR (cm $^{-1}$ Nujol): 3128, 1624, 1249, 700.

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.83–0.95 (t, 6H, $–CH_3$), 1.20–1.41 (m, 4H, $–CH_2–CH_3$), 1.60–1.82 (m, 4H, $–CH–CH_2$), 4.10–4.17 (quintet, 1H, Ar–H), 7.53–7.76 (dd, 3H, $J = 2$ Hz, $J = 5$ Hz, Ar–H), 8.21–8.30 (dd, 2H, $J = 8$ Hz, $J = 14$ Hz, Ar–H).

Anal. Calcd. for $C_{16}H_{19}ClN_4S$: C, 57.39; H, 5.72; N, 16.73; S, 9.58 **Found:** C, 57.24; H, 5.64; N, 16.83; S, 9.42.

*5.2. Crystal structure analysis of **2b***

A single crystal of $C_{11}H_9ClN_4S$ having dimensions of 0.2 × 0.2 × 0.2 mm was chosen for X-ray diffraction studies. The measurements were made on a Rigaku AFC7S diffractometer with graphite monochromated radiation (MoK_α). The data were collected by the ω-2θ scan technique and reduced using the teXsan data reduction program [17]. Lorentz and po-

larization corrections were applied. The structure was solved using direct methods, SHELXS-97 [18] and refined by least-squares method, SHELXL-97 [19].

5.3. Biology: in vitro evaluation of antimicrobial activity

Bacteria and fungal species used were obtained from Department of Studies in Microbiology, University of Mysore, India, namely, *Bacillus subtilis, Escherichia coli, Pseudomonas fluorescens, Xanthomonas campestris pvs, Xanthomonas oryzae, Aspergillus niger, Aspergillus flavus, Fusarium oxysporum, Trichoderma* sp. and *Fusarium monaliforme*. The bacterial strains were maintained on LB agar medium and the filamentous fungi were maintained on potato dextrose agar (PDA) medium at 28 °C. The disk diffusion method [20] was used to determine antibacterial and antifungal activity of synthesized compounds. Paper discs with DMSO were used as negative controls. The bacteria were grown in LB broth, centrifuged at 10,000 rpm for 5 min, pellet was dissolved in double distilled and used to inoculate the plates. For the filamentous fungi, the inoculum was prepared with the spores derived from 5–15 days' culture on PDA medium. The mycelia were covered with 10 ml of distilled water and the conidia were scraped using a sterile pipette. The spores were recovered after filtration on sterile absorbent cotton and were resuspended in sterile distilled water. The cell density of each inoculum was adjusted with hemocytometer in order to obtain a final concentration of approximately 10^4 CFU ml^{-1} and 10^6 spores per ml for the bacteria and filamentous fungi, respectively. Nystatin (Himedia) was used as a positive control for fungi and streptomycin and tetracycline for bacteria. Each disk contained 10 μg of standard drugs and 25 μg synthesized compounds. Plates were first kept at 4 °C for at least 2 hours to allow the diffusion of chemicals and then incubated at 28 °C. Inhibition zones were measured after 24 hours of incubation for bacteria and after 48 hours of incubation for fungi. The microdilution method [21] was followed to determine the minimum inhibitory concentration (MIC) of all the compounds against bacterial strains. The nutrient liquid medium was used as test media. Tests were performed in 96-well round bottom sterile culture plates. The wells of the microdilution plate were inoculated with 180 ml of the culture medium containing a final inoculum of $0.5 \times 2.5 \times 10^3$ CFU ml^{-1}. All the compounds previously solubilized in DMSO were serially diluted to two folds in the liquid medium and had concentration between 640–0.1 μg ml^{-1}. 20 μl of each concentration were added to each well containing the culture suspension except the growth control well. The final concentration ranged from 64 to 0.01 μg ml^{-1}. Plates were incubated at 35 °C for 48 hours. Growth was assessed at 494 nm by measuring the optical density in each well using an enzyme immunoassay multiwell reader (Sigma Diagnostic). Turbidometric method [22,23] was used to check antifungal activity of the compounds at different concentrations using nystatin as the positive control and DMSO as the negative control. To the culture tubes containing 1.9 ml of sterile media, 0.1 ml of the test compound was added at sterile conditions. Fresh inoculum was added to all the tubes including standard and controls with a spore concentration adjusted to 1×10^6 spores per ml. After incubating all tubes at 37 °C for 48 hours, absorbance was recorded at 610 nm. Percentage of inhibition was calculated according to the formula.

$$\% \text{ Inhibition} = 100(P - Q)/P$$

where P = absorbance without test sample and Q = absorbance with test sample. Then the MIC was recorded in μM. All determinant tests were performed duplicate and the results were reported as mean of these values.

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References

[1] F. Kurtzer, A.R. Katritzky, A.J. Boulton, in: Advances in Heterocyclic Chemistry, Academic Press, New York, 1965, pp. 165–209 (5).
[2] B. Shivarama Holla, K. Narayana Poojary, B. Sooryanarayana Rao, M.K. Shivananda, Eur. J. Med. Chem. 37 (2002) 511–517.
[3] K. Cooper, J. Steele, EP 329357, Chem Abstr 112 (1990) 76957.
[4] N.D. Heindel, J.R. Reid, J. Heterocycl. Chem. 17 (1980) 1087–1088.
[5] Z.Y. Zhang, S. Xiago-Wen, Heterocycles 48 (1998) 561–584.
[6] J.T. Witkoaski, R.K. Robins, R.W. Sidwell, L.N. Simon, J. Med. Chem. 15 (1972) 150–154.
[7] T. Tsukuda, Y. Shiratori, M.H. Watanabe, K. Ontsuka, M. Hattori, N. Shirai, Shimma, Bioorg. Med. Chem. Lett. 8 (1998) 1819–1824.
[8] H. Golgolab, I. Lalezari, L. Hosseini, Gohari, J. Heterocycl. Chem. 10 (1973) 387–390 (Chem Abstr. 79 (1973) 92159r).
[9] Basappa, M.P. Sadashiva, K. Mantelingu, S. Nanjunda Swamy, K.S. Rangappa, Bioorg. Med. Chem. 11 (2003) 4539–4544.
[10] Basappa, M. Satish Kumar, S. Nanjunda Swamy, M. Mahendra, J. Shashidhara Prasad, B.S. Viswanath, K.S. Rangappa, Bioorg. Med. Chem. Lett. 14(14) (2004) 3679–3681.
[11] B.S. Priya, Basappa, S. Nanjunda Swamy, Kanchugarakoppal S. Rangappa, Bioorg. Med. Chem. 13 (2005) 2623–2628.
[12] Shivananju Nanjunda Swamy, Basappa, Gangadharaiah Sarala, Basappa Prabhswamy, Sridhar. M. Anandalwar, Javaregowda. Shashidhara Prasad, Kanchugarakoppal. S. Rangappa, J. Chem. Res (S), 357 (2005) 291–298.
[13] M.P. Sadashiva, H. Mallesha, N.A. Hitesh, K.S. Rangappa, Bioorg. Med. Chem. 15; 12(24) (2004) 6389–6395.
[14] B. Prabhuswamy, S.Y. Ambekar, Synth. Commun. 29 (1999) 3487.
[15] T. George, R. Tahilramani, D.A. Dabholkar, Ind. J. Chemistry 5 (1969) 959–963.
[16] C.K. Johnson, ORTEP-II, A Fortran Thermal-Ellipsoid Plot Program, Report ORNL-5138, Oak Ridge National Laboratory, Oak Ridge, Tennessee, USA, 1976.
[17] TeXsan, Crystal Structure Analysis Package, Molecular Structure Corporation, Texas, USA, 1992.
[18] G.M. Sheldrick, SHELXS-97, University of Gőttingen, Germany, 1997.

[1] Full Crystallographic details deposited at Cambridge Crystallographic Database Center (CCDC no for compound **2b**: 260200). Copies of the data can be obtained by free of charge, on application to CCDC, 12 Union road, Cambridge, CB2 IEZ, UK (fax: +44(0) 1223336033 or e-mail: deposit@ccdc.cam.ac.uk).

[19] G.M. Sheldrick, SHELXS-97, University of Gőttingen, Germany, 1997.
[20] S. Lemriss, B. Marquet, H. Ginestet, L. Lefeuvre, A. Fassouane, P. Boiron, J. Mycol. Med. 13 (2003) 189–192.
[21] J.R. Zgoda, J.R. Porter, Pharmaceutical Biology. 39 (2001) 221–225.
[22] G. Barbaro, A. Battaglia, A. Dondoni, J. Chem. Soc. B (**1970**) 588.
[23] B.G. Mullen, R.T. Decory, T.J. Mitchell, D.S. Allen, C.R. Kinsolving, St. Vassil Georgiev, J. Med. Chem. 31 (1988) 2008.
[24] V.K. Chadha, G.R. Sharma, J. Ind. Chem. Soc. (1980) 1112–1114.