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Synthesis and biological evaluation of 2,4-disubstituted-[1,3]-thiazoles

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ABSTRACT

In the present study a series of novel 2,4-disubstituted-[1,3]-thiazole derivatives were synthesized by the reaction of 2-chloro-6-fluoro/2-fluorobenzaldehyde thiosemicarbazone with phenacyl bromide. 2-chloro-6-fluoro/2-fluorobenzaldehyde thiosemicarbazone was obtained by treating aldehyde thiosemicarbazone with thiosemicarbazide in the presence acetic acid in ethanol under reflux. Structures of newly synthesized compounds were characterized by spectral studies. New compounds were screened for their antimicrobial study and cytotoxic study. The results revealed that many of the synthesized thiazoles have good cytotoxicity and antimicrobial activity.

Key words: [1,3]-Thiazoles, cytotoxicity, antimicrobial activity.

INTRODUCTION

Thiazole is an important scaffold in heterocyclic chemistry and [1,3]-thiazole ring is present in many pharmacological active substances [1]. Thiazole derivatives have attracted a great deal of interest owing to their antimicrobial [2,3], anti-inflammatory [4,5], CNS depresent [6], antitubercular [7], antitumor [8], anthelmintic [9], sedative [10] antiretroviral properties [11], antineoplastic [12] activities as well as inhibitory activity of growth of gastrointestinal [13,14] and pancreatic adenocarcinoma cells [15]. In addition to being used in the pharmaceutical industry, thiazoles also find wide application in the dye and photographic industries. Encouraged by the above reports, it was planned to synthesize new [1,3]-thiazole derivatives containing chloro/fluorophenyl moiety at position 2 and substituted aryl group at position 4 of the thiazole ring, with the hope that the resulting molecules would exhibit promising biological properties. The present study describes the synthesis of unreported 2,4-disubstituted-[1,3]-thiazoles (**3a-l**) and evaluated their cytotoxicity and antimicrobial activity against pathogenic strains.

EXPERIMENTAL SECTION

Melting points were determined by the open capillary method and are uncorrected. The IR spectra (in KBr pellets) were recorded on a Shimadzu FT-IR 4100 type A spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian 400 MHz NMR spectrometer/Perkin-Elmer EM 300 MHz spectrometer using TMS as an internal standard. The mass spectra were recorded on a MDS SCIEX/API4000 spectrophotometer. Elemental analysis was carried out using Flash EA 1112 Series, CHNSO Analyzer (Thermo). The progress of the reaction was monitored by thin layer chromatography (TLC) on pre-coated silica gel plates. All the reagents used are from Spectrochem and Aldrich and used directly without any purification.

General Procedure for the Synthesis 2-(2-Chloro-6-fluoro/2-fluorobenzyledene) hydrazinecarbothioamide (2a,b)
An equimolar mixture of 2-chloro-6-fluoro/2-fluorobenzaldehyde (**79a**,**b**) (0.15 mol) and thiosemicarbazide (0.15 mol) and catalytic amount of acetic acid in ethanol (25 mL) was refluxed for 3 h. The solid thus obtained was filtered, dried and recrystallized using methanol/ethanol.

2-(2-Chloro-6-fluorobenzyledene)hydrazinecarbothioamide 2a
IR (KBr, $\gamma_{max,}$ cm^{-1}); 3406, 3230, 3200 (NH_2/NH), 3108 (Ar-H), 1594 (C=N), 1530 (C=C), 1233 (C=S), 1092 (C-F), 772 (C-Cl); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm); 7.23-7.44 (m, 4H, CH=N and 2-chloro-6-fluorophenyl-H), 8.28 (s, 2H, NH_2), 11.71 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 116.02, 120.81, 126.66, 131.94, 134.04, 135.98, 159.49, 178.77; DEPT (100 MHz, $CDCl_3$, δ ppm): 116.01, 126.66, 131.94, 135.98; MS (m/z): 232.4 (M^+); Anal. calcd. for $C_8H_7ClFN_3S$: C, 41.47; H, 3.05; N, 18.14; Found: C, 41.45; H, 3.00; N, 18.12.

2-(2-Fluorobenzyledene)hydrazinecarbothioamide 2b
IR (KBr, $\gamma_{max,}$ cm^{-1}); 3429, 3252, 3148 (NH_2/NH), 3013, 2977 (C-H), 1591 (C=N), 1515 (C=C), 1282 (C=S), 1060 (C-F); ^{1}H NMR (400 MHz, DMSO-*d6*, δ ppm); 7.16-7.43 (m, 3H, 2-fluorophenyl-H), 8.06 (s, 1H, -CH=N), 8.20 (t, 1H, 2-fluorophenyl-H, *J*=8.0), 8.26 (s, 2H, NH_2), 11.55 (s, 1H, NH), ^{13}C NMR: (100 MHz, DMSO-*d6*, δ ppm): 116.11, 122.15, 125.13, 127.26, 132.09, 132.18, 135.16, 160.02, 178.59; DEPT (100 MHz, $CDCl_3$, δ ppm): 116.31, 125.10, 127.26, 132.18, 135.16; MS (m/z): 198.2 (M+1); Anal. calcd. for $C_8H_8FN_3S$: C, 48.72; H, 4.09; N, 21.30; Found: C, 48.70; H, 4.05; N, 21.30.

General Procedure for the Synthesis of 2,4-Disubstituted-[1,3]-thiazoles (3a-l)
An equimolar mixture of 2-chloro-6-fluoro/2-fluorobenzyledene) hydrazinecarbothioamide (**2a**,**b**) (0.01 mol) and substituted phenacyl bromides (0.01 mol) in ethanol was refluxed for 4 h. After completion of the reaction, it was allowed to cool to room temperature. The solid thus separated was collected by filtration and recrystallized using methanol/ethanol.

1-(2-Chloro-6-fluorobenzylidene)-2-[4-(2,4-dichlorophenyl)-[1,3]-thiazol-2-yl]hydrazine 3a
IR (KBr, $\gamma_{max,}$ cm^{-1}); 3342 (NH), 3041, 2914 (C-H), 1598 (C=N), 1559 (C=C), 1099 (C-S), 1027 (C-F), 779 (C-Cl); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm); 7.26-7.48 (m, 5H, 2,4-dichlorophenyl/2-chloro-6-fluorophenyl-H), 7.57 (s, 1H, thiazolyl-H), 7.86 (d, 1H, 2,4-dichlorophenyl-H, *J*=8.5 Hz), 8.27 (s, 1H, -CH=N), 11.77 (s, 1H, NH); ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm): 110.32, 116.06, 120.93, 126.72, 127.95, 130.17, 131.24, 132.03, 132.39, 132.67, 133.03, 133.39, 134.63, 146.25, 161.85, 167.61; MS (m/z): 401.7 (M+1); Anal. calcd. for $C_{16}H_9Cl_3FN_3S$: C, 47.96; H, 2.26; N, 10.49; Found: C, 47.95; H, 2.25; N, 10.45.

5-{2-[2-(2-Chloro-6-fluorobenzylidene)hydrazino]-[1,3]-thiazol-4-yl}-2-hydroxybenzamide 3b
IR (KBr, $\gamma_{max,}$ cm^{-1}); 3340 (NH/OH), 3042, 2999 (C-H), 1698 (C=O), 1593 (C=N), 1560 (C=C), 1090 (C-S), 1025 (C-F), 748 (C-Cl); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 7.27-7.31 (m, 1H, 2-chloro-6-fluorophenyl-H), 7.37 (d, 1H, 2-chloro-6-fluorophenyl-H, *J*=8.5 Hz), 7.42 (t, 1H, 2-chloro-6-fluorophenyl-H, *J*=8.0 Hz), 7.57 (s, 1H, thiazolyl-H), 7.83 (d, 1H, salicylamide, *J*=8.7 Hz), 7.88 (s, 1H, salicylamide), 7.93 (d, 1H, salicylamide, *J*=8.7 Hz), 7.98 (s, 2H, NH_2), 8.36 (s, 1H, N=CH), 8.48 (s, 1H, OH), 12.62 (s, 1H, NH); MS (m/z): 391.87 (M+1); Anal. calcd. for $C_{17}H_{12}ClFN_4O_2S$: C, 52.24; H, 3.09; N, 14.34; Found: C, 52.23; H, 3.00; N, 14.30.

1-(2-Chloro-6-fluorobenzylidene)-2-[4-(1-oxo-1H-coumarin-3-yl)-[1,3]-thiazol-2-yl]hydrazine 3c
IR (KBr, $\gamma_{max,}$ cm^{-1}); 3352 (NH), 3005 (C-H), 1717 (C=O), 1599 (C=N), 1570 (C=C), 1120 (C-S), 1050 (C-F), 762 (C-Cl); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 6.99-7.06 (m, 4H, coumarinyl-H), 7.07-7.11 (m, 1H, 2-chloro-6-fluorophenyl-H), 7.13 (d, 1H, 2-chloro-6-fluorophenyl-H, *J*=8.0 Hz), 7.46 (t, 1H, 2-chloro-6-fluorophenyl-H, *J*=8.0 Hz), 7.52 (s, 1H, thiazolyl-H), 8.13 (s, 1H, coumarinyl-H), 8.42 (s, 1H, N=CH), 12.66 (s, 1H, NH); Anal. calcd. for $C_{19}H_{11}ClFN_3O_2S$: C, 57.08; H, 2.77; N, 10.51; Found: C, 57.06; H, 2.75; N, 10.52.

1-(2-Chloro-6-fluorobenzylidine)-2-[4-(2,5-dichloro-3-thienyl)-[1,3]-thiazol-2-yl]hydrazine 3e
IR (KBr, $\gamma_{max,}$ cm^{-1}); 3430 (NH), 2998 (C-H), 1572 (C=N), 1555 (C=C), 1127 (C-S), 1009 (C-F), 747 (C-Cl); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 7.29-7.36 (m, 1H, 2-chloro-6-fluorophenyl-H), 7.39 (d, 1H, 2-chloro-6-fluorophenyl-H, *J*=8.0 Hz), 7.46 (t, 1H, 2-chloro-6-fluorophenyl-H, *J*=8.0 Hz), 7.63 (s, 1H, thiazolyl-H), 7.92 (s, 1H, dichlorothiophenyl-H), 8.25 (s, 1H, CH=N), 12.52 (s, 1H, NH); ^{13}C NMR-DEPT (100 MHz, DMSO-d_6, δ ppm): 111.18, 115.9, 116.13, 126.73, 130.02, 131.4, 131.5, 135.37; MS (m/z): 407.8 (M+1); Anal. calcd. for $C_{14}H_7Cl_3FN_3S_2$: C, 41.34; H, 1.73; N, 10.33; Found: C, 41.32; H, 1.70; N, 10.30.

1-(2-Fluorobenzylidene)-2-[4-(2,4-dichlorophenyl)-[1,3]-thiazol-2-yl]hydrazine 3g
IR (KBr, $\gamma_{max,}$ cm^{-1}); 3498 (NH), 3098 (C-H), 1633 (C=N), 1571 (C=C), 1106 (C-S), 1031 (C-F), 748 (C-Cl); ^{1}H NMR (400 MHz, DMSO-*d6*, δ ppm): 7.05-7.40 (m, 5H, 2-fluorophenyl/2,4-dichlorophenyl-H), 7.47 (s, 1H, thiazolyl-H), 7.68 (d, 1H, 2,4-dichlorophenyl-H, *J*=8.4), 7.88 (t, 1H, 2,4-dichlorophenyl-H, *J*=7.4 Hz), 8.24 (s, 1H, CH=N), 11.69 (s, 1H, NH); ^{13}C NMR-DEPT (100 MHz, DMSO-*d6*, δ ppm): 108.14, 116.18, 124.47, 126.82, 127.83, 130.66, 131.37, 132.30, 140.76; MS (m/z): 368.0 (M+1); Anal. calcd. for $C_{16}H_{10}Cl_2FN_3S$: C, 52.47; H, 2.75; N, 11.47; Found: C, 52.45; H, 2.70; N, 11.45.

5-{2-[2-(2-Fluorobenzylidene)hydrazino]-[1,3]-thiazol-4-yl}-2-hydroxybenzamide 3h
IR (KBr, $\gamma_{max,}$ cm^{-1}); 3440 (NH/OH), 3012 (C-H), 1689 (C=O), 1600 (C=N), 1566 (C=C), 1098 (C-S), 1029 (C-F); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 7.03-7.06 (m, 1H, 2-fluorophenyl-H), 7.10 (d, 1H, 2-fluorophenyl-H, *J*=8.2 Hz), 7.43 (t, 1H, 2-fluorophenyl-H, *J*=7.4 Hz), 7.47 (s, 1H, thiazolyl-H), 7.85 (d, 1H, salicylamide, *J*=8.7 Hz), 7.88 (s, 1H, salicylamide), 7.92 (t, 1H, 2-fluorophenyl-H, *J*=7.5 Hz), 7.95 (d, 1H, salicylamide, *J*=8.7 Hz), 7.99 (s, 2H, NH_2), 8.38 (s, 1H, CH=N), 8.49 (s, 1H, OH), 12.65 (s, 1H, NH), MS (m/z): 357.5 (M+1); Anal. calcd. for $C_{17}H_{13}FN_4O_2S$: C, 57.29; H, 3.68; N, 15.72; Found: C, 57.23; H, 3.66; N, 15.70.

1-(2-Fluorobenzylidine)-2-[4-(1-oxo-1H-coumarin-3-yl)-[1,3]-thiazol-2-yl]hydrazine 3i
IR (KBr, $\gamma_{max,}$ cm^{-1}); 3430 (NH), 2998 (C-H), 1715 (C=O), 1590 (C=N), 1571 (C=C), 1105 (C-S), 1053 (C-F); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 6.98 (m, 4H, coumarinyl-H), 7.05-7.09 (m, 1H, 2-fluorophenyl-H), 7.10 (d, 1H, 2-fluorophenyl-H, *J*=8.4 Hz), 7.42 (t, 1H, 2-fluorophenyl-H, *J*=7.5 Hz), 7.49 (s, 1H, thiazolyl-H), 7.92 (t, 1H, 2-fluorophenyl-H, *J*=7.6 Hz), 8.11 (s, 1H, coumarinyl-H), 8.39 (s, 1H, N=CH), 12.63 (s, NH); Anal. calcd. for $C_{19}H_{12}FN_3O_2S$: C, 62.46; H, 3.31; N, 11.50; Found: C, 62.44; H, 3.30; N, 11.52.

1-(2-Fluorobenzylidine)-2-[4-(2,5-dichloro-3-thienyl)-[1,3]-thiazol-2-yl]hydrazine 3k
IR (KBr, $\gamma_{max,}$ cm^{-1}); 3433 (NH), 2990 (C-H), 1570 (C=N), 1531 (C=C), 1140 (C-S), 1087 (C-F), 760 (C-Cl); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm); 7.27 (d, 1H, 2-fluorophenyl-H, *J*=8.5 Hz), 7.43-7.48 (m, 1H, 2-fluorophenyl-H), 7.65 (t, 1H, 2-fluorophenyl-H, *J*=7.5 Hz), 7.74 (s, 1H, thiazolyl-H), 7.84 (t, 1H, 2-fluorophenyl-H, *J*=7.6 Hz), 7.95 (s, 1H, dichlothiophenyl-H), 8.20 (s, 1H, CH=NH), 12.40 (s, 1H, NH); Anal. calcd. for $C_{14}H_7Cl_3FN_3S_2$: C, 41.34; H, 1.73; N, 10.33; Found: C, 41.32; H, 1.70; N, 10.30.

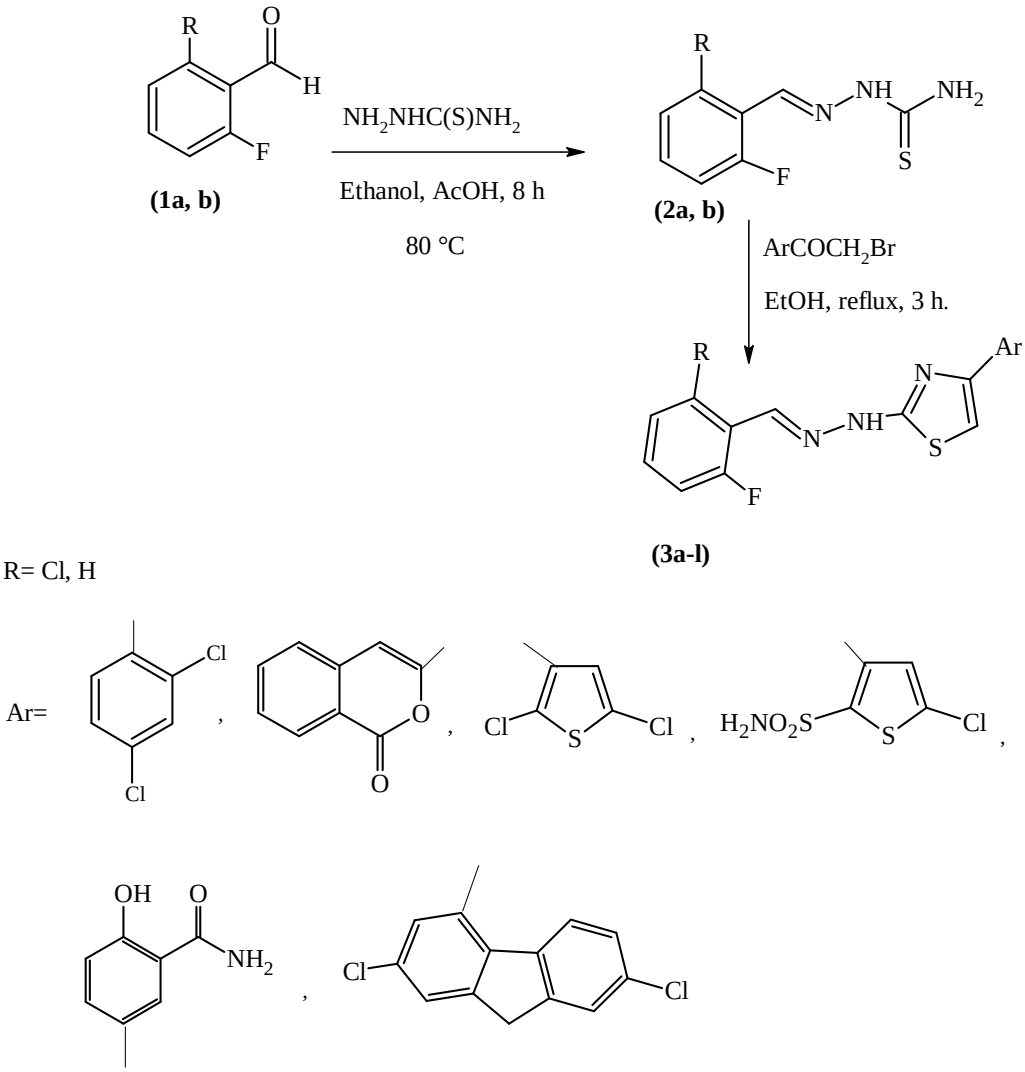
RESULTS AND DISCUSSION

The targeted thiazoles (**3a-l**) were obtained in good yield by refluxing substituted thiosemicarbazones (**2a,b**) with various phenacylbromides in ethanol for 8 h. The starting material (**2a,b**) in turn was synthesized by refluxing equimolar amount of 2-chloro-6-fluoro/2-fluorobenzaldehyde with thiosemicarbazide in the presence of acetic acid in ethanol. The reaction pathway has been summarized in **Scheme 1**. Newly synthesized compounds (**3a-l**) were characterized by IR, NMR, mass spectral and C, H, N elemental analyses. The characterization data of synthesized compounds are presented in **Table 1.**

The intermediate compound (**2a,b**) was confirmed by its spectral data**.** The IR spectrum of **2a** showed the absorption bands corresponding to NH_2/NH at 3406, 3230 and 3200 cm^{-1}. The band at 3108 cm^{-1} was due to Ar-H stretching vibration. The absorption band at 1594cm^{-1} was due to C=N stretching vibration. The absorption band appearing at 1233 cm^{-1} was due to C=S bond. The absorption bands appearing at 772 and was due to C-Cl stretching and the absorption band seen at 1092cm^{-1} was due to C-F stretching. The ^{1}H NMR spectrum of **2a** showed a sharp singlet at δ11.71 corresponding to NH proton. Three protons of 2-chloro-6-fluorophenyl ring and one NH proton resonated as a multiplet in the region δ7.23-7.44. The signal due to NH_2 protons were appeared as a singlet at δ8.28. The 100 MHz ^{13}C NMR spectrum showed characteristic signals at δ116.02, 120.81, 126.66, 131.94, 134.04, 135.98, 159.49, 178.77. ^{13}C NMR DEPT spectrum showed four signals for the non quaternary carbon atoms. Further, Mass spectrum of **2a** showed a (M^+) peak at *m/z* 232.4 corresponding to its molecular formula, $C_8H_7ClFN_3S$.

The IR spectrum of compound **2b** showed the absorption band corresponding to NH_2/NH groups at 3429, 3252 and 3148 cm^{-1}. The bands at 3013 and 2977 cm^{-1} were due to C-H stretching vibration. The absorption band at 1591cm^{-1} was due to C=N bond. The absorption band seen at 1282 cm^{-1} was due to C=S bond. The C-F absorption band appeared at 1060 cm^{-1}. The ^{1}H NMR spectrum showed a sharp singlet at δ11.55 corresponding to NH proton. Two protons of NH_2 resonated as a distinct singlet at δ8.26. Four protons of 2-fluorophenyl ring and a proton of CH=N

group resonated as multiplet in the region δ 7.16-8.22. The 100 MHz ^{13}C NMR spectrum of **2b** showed characteristic signals at δ 116.32, 122.15, 125.13, 127.26, 132.09, 132.18, 135.16, 160.02, 178.59 thereby accounting for the presence of eight carbon atoms in the molecule. Further, MS spectrum showed a (M+1) peak at *m/z* 198.2 corresponding to its molecular formula, $C_8H_8FN_3S$.



Scheme 1 Synthesis of [1,3]-thiazoles

The IR spectrum of **3a** showed the absence of absorption bands corresponding to NH_2 group. The absorption band at 1598cm^{-1} was due to C=N bond. The absorption band seen at 1099 cm^{-1} was due to C-S stretching. The absorption bands due to C-F and C-Cl stretching vibrations were observed at 1027 and 779 cm^{-1} respectively. The ^{1}H NMR spectrum of showed a singlet at δ8.27 corresponding to CH=N proton. Two protons of 2-chloro-6-fluorophenyl and three protons of 2,4-dichlorophenyl rings were resonated as a multiplet at δ7.26-7.48. One proton of 2-chloro-6-fluorophenyl ring resonated as a doublet at δ7.86 (*J*=8.5 Hz). The 100 MHz ^{13}C NMR spectrum of **3a** showed characteristic signals at δ110.32, 116.06, 120.93, 126.72, 127.95, 130.17, 131.24, 132.03, 132.39, 132.67, 133.03, 133.39, 134.63, 146.25, 161.82 and 167.61. Further, Mass spectrum showed a (M+1) peak at *m/z* 401.7 corresponding to its molecular formula, $C_{16}H_9Cl_3FN_3S$.

The IR spectrum of thiazolyl hydrazine derivative **3g** showed the absorption bands corresponding to NH and Ar-H at 3498 and 3098 cm^{-1} respectively. The absorption band seen at 1571cm^{-1} was due to C=N. The absorption band corresponding to C-S stretching was observed at 1106 cm^{-1}. C-F and C-Cl stretching bands were observed at 1031 and 748 cm^{-1} respectively. The ^{1}H NMR spectrum of **3g** showed a singlet at δ 8.24 corresponding to N=CH proton. Two protons of 2,4-dichlorophenyl ring resonated as a triplet at δ 7.88 (J=7.4 Hz) and a doublet at δ 7.68 (J=8.4 Hz). A singlet at δ 7.47 was due to thiazole ring proton. Four protons of 2-fluorophenyl ring and one proton of 2,4-dichlorophenyl ring resonated as a complex multiplet in the region δ 7.05-7.40. The 100 MHz ^{13}C NMR DEPT spectrum of **3g** showed characteristic signals at δ 108.14, 116.18, 124.47, 126.82, 127.83, 130.66, 131.37, 132.30 and 140.76. Further, Mass spectrum of **3g** showed a (M+1) peak at *m/z* 368.0 corresponding to its molecular formula, $C_{16}H_{10}Cl_2FN_3S$.

Biological activity

Cytotoxicity

Procedure for MTT assay: The cytotoxicity study was performed by the MTT assay [16]. The MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazollium bromide] assay was performed on HeLa cells (human epithelial cervical cancer). Selected healthy cell lines with specified concentrations were harvested. Stock solutions were prepared for each test samples by dissolving in DMSO or DMF and made up to a concentration of 20 mg/mL. Serial dilutions were carried out to give four different sub-stocks with concentrations of 200.00, 100.00, 50.00 and 25.00 μg/mL.

Table 1 Characterization data of [1,3]-thiazoles 3a-l

Compd.	R	Ar	Mol. Formula	Mol. Wt.	mp (°C)	Yield (%)
2a	Chloro	-	$C_8H_7ClFN_3S$	231.37	216-218	80%
2b	H	-	$C_8H_8FN_3S$	197.23	177-180	80%
3a	Chloro	2,4-Cl_2-C_6H_3-	$C_{16}H_9Cl_3FN_3S$	400.68	162-164	80%
3b	Chloro	4-$CONH_2$-5-OH-C_6H_3-	$C_{17}H_{12}ClFN_4O_2S$	390.81	dec 237	82%
3c	Chloro	3-coumarinyl	$C_{19}H_{11}ClFN_3O_2S$	399.82	257-259	90%
3d	Chloro	2-SO_2NH_2-5-Cl-3-thienyl	$C_{14}H_9Cl_2FN_4O_2S_3$	451.34	256-258	89%
3e	Chloro	2,5-Cl_2-3-thienyl	$C_{14}H_7Cl_3FN_3S_2$	406.71	253-258	90%
3f	Chloro	2,7-Cl_2-*9H*-4-fluorenyl	$C_{23}H_{13}Cl_3FN_3S$	488.79	253-255	95%
3g	H	2,4-Cl_2-C_6H_3-	$C_{16}H_{10}Cl_2FN_3S$	366.24	168.171	82%
3h	H	4-$CONH_2$-5-OH-C_6H_3-	$C_{17}H_{13}FN_4O_2S$	356.37	dec 216	83%
3i	H	3-coumarinyl	$C_{19}H_{12}FN_3O_2S$	365.28	240-242	89%
3j	H	2-SO_2NH_2-5-Cl-3-thienyl	$C_{14}H_{10}ClFN_4O_2S_3$	416.90	265-266	88%
3k	H	2,5-Cl_2-3-thienyl	$C_{14}H_8Cl_2FN_3S_2$	372.26	260-262	92%
3l	H	2,7-Cl_2-*9H*-4-fluorenyl	$C_{23}H_{14}Cl_2FN_3S$	454.34	dec 243	92%

An aliquot of 100 μL of each sub-stock with different concentrations were added to each well together with 100 μL of selected cells to give concentrations of 100.00, 50.00, 25.00 and 12.50 μg/mL and made up to a final volume of 200 μL in each well. Cells with no test samples (200 μL, untreated cell control, positive control) and 200 μL of medium only (blank medium, negative control) were prepared in the same plate. Samples and controls were prepared in triplicate. The plate was then incubated for 72 h at 37 °C in 5% CO2 incubator. After 72 h, 20 μL of MTT solution was added to all the wells and incubated for 3 h in a 5% CO2 incubator. The plate was then spun at 3,000 rpm for 10 min. Supernatant from each well (160 μL) was discarded and then DMSO (160 μL) was added to dissolve the purple formazan crystals. The absorbance of each well was determined using a microplate reader at 550 nm. The average absorbance of each test samples by dissolving in DMSO or DMF and made up to a concentration of 20 mg/mL. Serial dilutions were carried out to give six different sub-stocks with concentrations of 200.00, 100.00, 50.00 and 25.00 μg/mL. An aliquot of 100 μL of each sub-stock with different concentrations were added to each well together with 100 μL of selected cells to give concentrations of 100.00, 50.00, 25.00 and 12.5 μg/mL was calculated and the average value was used to determine the percentage of cell death by using the following formula:

$$\% \text{ cell death} = \frac{\text{Absorbance}_{(\text{-ve control})} - \text{Absorbance}_{(\text{sample})}}{\text{Absorbance}_{(\text{-ve control})} - \text{Absorbance}_{(\text{+ve control})}} \times 100$$

A graph of percentage of cell death versus concentration was plotted for each sample. The IC50 values were obtained from the plotted graph. Further dilutions were only performed on the compounds with IC50 values less than 6.25 μg/mL. Three independent experiments were conducted to assure the accuracy of the results. Doxorubicin was used as standard compound throughout the cytotoxicity experiments.

Table 2 Cytotoxic activity data of [1,3]-thiazoles 3a-l

Compd.	Conc (μM)	% Cell death	Standard Error	IC_{50} (μM)
Doxorubicin	2.5	78.2	2.5	4.1
3a	12.5	20.14	4.65	42.1
	25	27.22	4.47	
	50	55.05	4.98	
	100	62.30	3.62	
3b	12.5	10.16	1.62	>100
	25	13.17	4.44	
	50	15.16	1.98	
	100	16.68	2.42	
3c	12.5	60.76	0.33	10.2
	25	70.76	2.39	
	50	85.73	0.17	
	100	90.05	1.77	
3d	12.5	75.70	2.82	8.3
	25	80.61	2.05	
	50	79.53	0.58	
	100	78.43	0.43	
3e	12.5	61.57	3.05	12.5
	25	67.73	2.05	
	50	76.27	2.21	
	100	72.22	0.47	
3f	12.5	7.61	2.76	>100
	25	7.7	8.39	
	50	8.02	2.68	
	100	10.2	4.58	
3g	12.5	21.49	4.73	44.1
	25	19.53	15.07	
	50	53.93	2.55	
	100	79.33	0.36	
3h	12.5	43.71	0.88	17.8
	25	55.98	2.49	
	50	59.92	3.59	
	100	76.02	3.18	
3i	12.5	38.39	0.77	82.4
	25	37.70	2.65	
	50	40.31	1.45	
	100	56.11	2.64	

Table 2 Cytotoxic activity data of [1,3]-thiazoles 3a-l continued

Compd.	Conc (μM)	% Cell death	Standard Error	IC_{50} (μM)
Doxorubicin	2.5	78.2	2.5	4.1
3j	12.5	59.60	0.63	10.5
	25	64.84	0.30	
	50	72.27	2.42	
	100	79.09	4.96	
3k	12.5	27.91	5.51	48.7
	25	33.01	1.17	
	50	50.48	3.58	
	100	54.09	1.88	
3l	12.5	08.60	5.66	>100
	25	13.12	2.15	
	50	20.90	2.57	
	100	26.39	2.89	

The results of the MTT assay on HeLa cells (human epithelial cervical cancer) showed that compound **3c**, **3d** and 3**e** containing 3-coumarinyl, 2-SO_2NH_2-5-Cl-3-thienyl, 2,5-Cl_2-3-thienyl groups respectively at C-4 position of [1,3]-thiazole derivatives were found to possess significant activity compared to the standard Doxorubicin. It was found that the derivatives [1,3]-thiazole containing 2-chloro-6-fluorophenyl were found to be more active than the

thiazoles containing compounds with 2-fluorophenyl moiety. The compounds with 2-fluorophenyl and 3-coumarinyl group showed good activity. Other compounds showed moderate to good activity. The results of cytotoxic bioassay studies are given in **Table 2**.

Antimicrobial activity

The newly synthesized [1,3]-thiazole (**3a-l**) were screened for their *in vitro* antibacterial and antifungal activity. For antibacterial studies micro-organisms employed were *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus cereus.* For antifungal screening, *Candida albicans* strain was used. Both microbial studies were assessed by Minimum Inhibitory Concentration (MIC) by serial dilution method [17]. For this, the compound whose MIC has to be determined was dissolved in serially diluted DMF. Then a standard drop of the culture prepared for the assay is added to each of the dilutions, and incubated for 16–18 h at 37 °C. MIC is the highest dilution of the compound, which shows clear fluid with no development of turbidity. The antimicrobial activity data are presented in **Table 3** and **Table 4**. Penicillin and Flucanazole were used as reference standards for antibacterial and antifungal activity respectively. In order to ensure that the solvent had no effect on bacterial growth, a negative control test also performed containing inoculated broth supplemented with only DMF at the same dilution used in our experiment and found inactive in culture media. Three replicates were made for each analysis. The MIC values were evaluated at concentration range, 0.024-50 µg/mL. The MIC was noted by seeing the lowest concentration of the drug at which there was no visible growth. The figures in the tables show the MIC values in µg/mL.

Table 3 Antibacterial activity data of [1,3]-thiazoles 3a-l

Compd.	MIC in µg/mL			
	E. coli	***S. aureus***	***P. aeruginosa***	***B. cereus***
3a	6.25	6.25	3.125	6.25
3b	3.125	6.25	3.125	6.25
3c	3.125	6.25	3.125	12.5
3d	3.125	25.0	3.125	3.125
3e	3.125	3.125	3.125	3.125
3f	3.125	3.125	3.125	3.125
3g	6.25	6.25	6.25	6.25
3h	3.125	6.25	6.25	6.25
3i	3.125	3.125	3.125	3.125
3j	6.25	6.25	6.25	6.25
3k	3.125	6.25	3.125	6.25
3l	3.125	3.125	3.125	3.125
Penicillin	0.097	0.097	0.097	0.097

Table 4 Antifungal activity data of [1,3]-thiazoles 3a-l

Compd.	MIC in µg/mL
	C. albicans
3a	6.25
3b	1.562
3c	3.125
3d	3.125
3e	6.25
3f	3.125
3g	6.25
3h	6.25
3i	3.125
3j	0.781
3k	3.125
3l	6.25
Flucanazole	0.781

The investigation of antimicrobial screening data revealed that all the tested compounds (**3a-l**) exhibit moderate to good antibacterial and antifungal activities against pathogenic strains. The compounds **3e**, **3f**, **3i** and **3l** have shown highest antibacterial activity against all the microbial strains used for screening. The significant activity against bacterial pathogens was also provided by the compounds **3b**, **3d** and **3k** at the concentration of 3.125 µg/mL. On the other hand, the compounds **3a**, **3h**, **3g** and **3j** exhibited moderate growth inhibition. Investigation of antifungal data revealed that compounds **3b**, **3c, 3d, 3e, 3f, 3h, 3i, 3j, 3k, 3l** exhibit significant activity against the fungal pathogens at the concentration of <0.3125 µg/mL. Compound **3j** exhibited good antifungal activity. Compounds **3a** and **3g** exhibited moderate growth inhibition at 6.25 µg/mL concentration against *C. albicans*.

From the antimicrobial activity results obtained, the structure activity relationship can be drawn for test compounds (**3a-l**). The variation in antimicrobial activity of the test compounds was explored by varying the substituent at C-2 and C-4. The thiazoles carrying 2-chloro-6-fluorophenyl moiety were found to be more active when compared with the other derivatives carrying 2-fluorophenyl moiety. The compounds **3e**, **3f**, **3i** and **3l** have better activity because of dichlorothienyl, chlorofluorenyl and 3-coumarinyl groups at C-4 position. In case of antifungal activity, **3j** having 2-fluorophenyl at position C-2 and 2-SO_2NH_2-5-Cl-3-thienyl group at C-4 of the thiazole ring possessed highest activity. Similarly the compound **3b** having 2-chloro-6-fluorophenyl at position C-2 and 4-$CONH_2$-5-OH-phenyl group at C-4 of the thiazole ring exhibited good growth inhibition. All the compounds except **3a** and **3g** showed low activity among the twelve compounds may be due to the presence of dichlorophenyl substituent at C-4 position of the thiazole.

CONCLUSION

This research study reports the successful synthesis of some 2,4-disubstituted-[1,3]-thiazoles (**3a-l**) in good yields. All the synthesized compounds have been characterized by analytical and spectral data. The cytotoxic study and antimicrobial activity evaluation of these compounds were done. Many of the synthesized thiazoles exhibited good cytotoxicity and antimicrobial activity.

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