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Synthesis, Anticancer and antibacterial Activities of Triazolothiadiazines Containing 2,4-dichloro-5-fluorophenyl Moiety

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ABSTRACT

*A new series of [1,2,4]-triazolo-[3,4-b][1,3,4]-thiadiazines (**3a-h**) possessing arylfurfuryl, 2,4-dichloro-5-fluorophenyl and aryloxymethyl/anilinomethyl moieties was prepared by the reaction of various 4-amino-5-((aryloxy/arylamino)methyl)-4H-[1,2,4]-triazole-3-thiols **(1a-h)** with 1-(2,4-dichloro-5-fluorophenyl)-3-arylfuryl-2-bromo-2-propen-1-ones **(2)** in the presence of potassium hydroxide. All the synthesized compounds were evaluated in vitro for their antibacterial activity and compound **3c** and **3h** showed excellent activity. Compound **3d** and **3h** were evaluated for anticancer screening study and showed promising activity against some of the cell lines. The newly synthesized compounds were confirmed by IR, ^{1}H NMR, and mass spectral analysis.*

Keywords: [1, 2, 4]-Triazoles; [1, 3, 4]-thiadiazines; 2,4-dichloro-5-fluorophenyl; antibacterial activity; anticancer activity.

INTRODUCTION

The structural diversity and biological importance of [1, 2, 4]-triazole containing heterocycles have made them attractive targets for synthesis over many years. Recently *N*-bridged heterocycles derived from [1, 2, 4]-triazole has drawn much attention in medicinal field as it has been found wide application in different theoretical areas [1]. [1, 2, 4]-Triazole scaffold is present in numerous drug candidates; incorporation of this nucleus has remarkably increased the efficacy of the drugs [2, 3]. [1, 2, 4]-Triazoles and their derivatives showed different biological activities such as antimicrobial [4, 5], anti-inflammatory [6], anticancer [7], anti-hypertensive [8], antitubercular [9], antidepressant [10] and antiviral [11].

Recently, introduction of fluorinated heterocycles in drug discovery is enhanced and nowadays fluorinated heterocycles are synthesized on a routine basis by medicinal chemists [12]. The ability of fluorine atom to act as polar hydrogen or hydroxyl mimic makes it a good strategy in designing new bio-molecules by the replacement of hydrogen atom [13]. Thus, fluorine substitution remains an attractive means in the development of more active and selective pharmaceutical drug molecules. The synthetic and pharmacological utility of 2,4-dichloro-5-fluoro phenyl moiety was reported by our group and it was found

that the presence of this halo aromatic system significantly enhances the biological activity of heterocycles [14-16].
Triazolothiadiazines is an interesting fused system derived from 5-substituted-4-amino-[1,2,4]-triazole-3-thiols. This fused system is found to be associated with diverse pharmacological activities [17, 18]. Moreover aryl furan-2-carboxaldehyde derivatives have been reported to possess wide spectrum of biological activities [19]. Recently we have reported the significant antimicrobial and anticancer properties of triazolo thiadiazines containing 2,4-dichloro-5-fluoro phenyl moiety [20, 21]. In the light of these observations and in continuation of our studies on N-bridged heterocycles derived from [1, 2, 4]-triazoles [22, 23], we undertook the synthesis of some newer congeners of triazolothiadiazines having aryloxy methyl/ anilinomethyl, 2,4-dichloro-5-fluorophenyl and aryl furan moieties in a single molecular framework. All the compounds were screened for their antibacterial activity and two compounds from this series were screened for their anticancer activities. Results of such studies are described in this paper.

MATERIALS AND METHODS

Melting points were determined in open capillaries and are uncorrected (melting point apparatus: SERWELL Instruments Inc., India). Purity of the compounds was checked by thin layer chromatography (TLC) on a silica coated aluminium sheet (silica gel 60F254) using chloroform and methanol (9:1, v/v). IR spectra were recorded on NICOLET AVATAR 330- FTIR Spectrometer. 1H NMR spectra recorded on a Varian 300 MHz NMR spectrometer using TMS as an internal standard. (Chemical shifts in δ ppm). The spin multplets are described as singlet (s), doublet (d), triplet (t), quartet (q), broad (br) and multiplet (m). The FAB mass spectra were recorded on a JEOL SX 102/DA-6000 spectrophotometer/Data system using argon/xenon (6 kV, 10 mA) FAB gas, at 70 eV. Elemental analysis was carried out using Flash EA 1112 Series, CHNSO Analyzer (Thermo). Solvents and reagents were purchased from the commercial vender's in the appropriate grade and were used without purification.

General procedure for the preparation of 7-arylfurylidene-6-(2,4-dichlorophenyl)-3-(aryloxy methyl/ anilinomethyl)-*7H*-[1,2,4]-triazolo[3,4-*b*][1,3,4]thiadiazine(3a-h):A mixture of 3-substituted-4-amino-5-mercapto-1,2,4-triazole **1** (0.01 mol), 1-(2,4-dichloro-5-fluorophenyl)-3-arylfuryl-2-bromo-2-propen-1-one **2** (0.01 mol) and ethanolic potassium hydroxide (10%, 2.5 mL) was kept under reflux on a water bath for about 5 h. After the completion of reaction, reaction mass was cooled to room temperature. The precipitated solid was filtered, washed with water and recrystalized from ethanol.

3-((2-Chlorophenoxy)methyl)-7-((5-(4-chlorophenyl)furan-2-yl)methylene)-6-(2,4-di- chloro-5-fluoro phenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine 3a: IR (KBr, cm^{-1}): 3010 (ArC-H), 2920 (C-H), 1630 (C=N), 1590 (C=C), 1090 (C-F), 840 (ArC-H def.), 731 (C-Cl). ^{1}H NMR(DMSO-d_6, δ ppm): 5.40 (2H, s, CH_2), 6.51 (1H, s, exocyclic =CH), 6.92 (1H, d, J = 3.7 Hz, furan C_3H), 7.33-7.39 (m, 5H, , Ar-H & furan C_4H), 7.40-7.50 (m, 4H, Ar-H), 7.63 (1H, d, J = 6.3 Hz, ArH), 8.07 (1H, d, J = 8.5 Hz, ArH), MS (m/z): 633 ($[M+H]^+$.

3-((4-Chlorophenoxy)methyl)-7-((5-(4-chlorophenyl)furan-2-yl)methylene)-6-(2,4-di- chloro-5-fluoro phenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine 3b: IR (KBr, cm^{-1}): 3013 (ArC-H), 2928 (C-H), 1632 (C=N), 1595 (C=C), 1089 (C-F), 741 (C-Cl). ^{1}H NMR(DMSO-d_6, δ ppm): 5.41 (2H, s, CH_2), 6.52 (1H, s, exocyclic =CH), 6.90 (1H, d, J = 3.7 Hz, furan C_3H), 7.30-7.38 (m, 5H, Ar-H & furan C_4H), 7.44-7.47 (m, 4H, Ar-H), 7.65 (1H, d, J = 8.5 Hz, ArH), 8.01 (1H, d, J = 6.3 Hz). MS (m/z): 633 ($[M+H]^+$.

3-((2,4-Dichlorophenoxy)methyl)-7-((5-(4-chlorophenyl)furan-2-yl)methylene)-6-(2,4-dichloro-5-fluorophenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine 3c: IR (KBr, cm^{-1}): 3020 (ArC-H), 2925 (C-H), 1635 (C=N), 1600 (C=C), 1090 (C-F), 750 (C-Cl). ^{1}H NMR(DMSO-d_6, δ ppm): 5.40 (2H, s, CH_2), 6.55 (1H, s, exocyclic =CH), 6.91 (1H, d, J = 3.7 Hz, furan C_3H), 7.32-7.37 (m, 4H, d, Ar-H & furan C_4H),

7.45-7.48 (m, 4H, Ar-H), 7.63 (d, 1H, *J* = 8.5 Hz, ArH), 8.04 (1H, d, *J* = 8.5 Hz, ArH). MS (m/z): 667 ($[M+H]^+$.

3-((4-Chloro-3-methylphenoxy)methyl)-7-((5-(4-chlorophenyl)furan-2-yl)methylene)-6-(2,4-dichloro-5-fluorophenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine 3d: IR (KBr, cm^{-1}): 3025 (ArC-H), 2925 (C-H), 1630 (C=N), 1590 (C=C), 1090 (C-F), 742 (C-Cl). ^{1}H NMR(DMSO-d_6, δ ppm): 5.41 (s, 2H, CH_2), 6.55 (s, 1H, exocyclic =CH), 6.91 (d, 1H, *J* = 3.7 Hz, furan C_3H), 7.14 (s, 1H, Ar-H), 7.33-7.47 (m, 4H, Ar-H & furan C_4H), 7.42 (d, 1H, J = 8.4Hz, ArH), 7.47 (d, 2H, 8.4Hz, Ar-H), 7.64 (d, 1H, *J* = 8.5 Hz, ArH), 8.02 (1H, d, *J* = 8.5 Hz, ArH). MS (m/z): 647 ($[M+H]^+$.

3-((3,4-Dimethylphenoxy)methyl)-7-((5-(4-chlorophenyl)furan-2-yl)methylene)-6-(2,4-dichloro-5-fluorophenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine 3e: IR (KBr, cm^{-1}): 3023 (ArC-H), 2922 (C-H), 1626 (C=N), 1597 (C=C), 1083 (C-F), 738 (C-Cl). ^{1}H NMR(DMSO-d_6, δ ppm): 5.40 (s, 2H, CH_2), 6.53 (s, 1H, exocyclic =CH), 6.95 (d, 1H, *J* = 3.7 Hz, furan C_3H), 7.09 (s, 1H, Ar-H), 7,11 (d, 1H, Ar-H0, 7.22 (d, 1H, Ar-H), 7.35-7.40 (m, 3H, Ar-H & furan C_4H), 7.52 (d, 2H, 8.4Hz, Ar-H), 7.65 (d, 1H, $J_{m\text{-}F}$ = 8.4Hz, ArH), 7.99 (d, 1H, *J* = 6.3 Hz, ArH). MS (m/z): 625 ($[M+H]^+$.

N-((7-((5-(4-Chlorophenyl)furan-2-yl)methylene)-6-(2,4-dichloro-5-fluorophenyl)-7H-[1,2,4]triazolo [3,4-b][1,3,4]thiadiazine-3-yl)methyl)-benzenamine 3f: IR (KBr, cm^{-1}):34001 (N-H), 3015 (ArC-H), 2918 (C-H), 1629 (C=N), 1594 (C=C), 1090 (C-F), 739 (C-Cl). ^{1}H NMR (DMSO-d_6, δ ppm): 4.91 (s, 1H, NH), 5.82 (s, 2H, N-CH_2), 6.72 (1H, s, exocyclic =CH), 7.00 (1H, d, *J* = 3.7 Hz, furan C_3H), 7.23-7.28 (m, 3H, Ar-H), 7.37-7.42 (m, 3H, Ar-H & furan C_4H), 7.53 (d, 2H, Ar-H, 8.5Hz), 7.65 (d, 1H, *J* = 8.5 Hz, ArH), 8.0 (d, 1H, *J* = 6.3 Hz, Ar-H). MS (m/z): 598 ($[M+H]^+$.

N-((7-((5-(4-Chlorophenyl)furan-2-yl)methylene)-6-(2,4-dichloro-5-fluorophenyl)-7H-[1,2,4]triazolo [3,4-b][1,3,4]thiadiazine-3-yl)methyl)-4-chlorobenzenamine 3g: IR (KBr, cm^{-1}):3400 (N-H), 3012 (ArC-H), 2919 (C-H), 1632 (C=N), 1592 (C=C), 1088 (C-F), 738 (C-Cl). ^{1}H NMR(DMSO-d_6, δ ppm): 4.90 (s, 1H, NH), 5.86 (s, 2H, N-CH_2), 6.74 (1H, s, exocyclic =CH), 7.03 (1H, d, *J* = 3.7 Hz, furan C_3H), 7.34 (1H, d, *J* = 3.7 Hz, furan C_4H), 7.38 (d, 2H, 8.4Hz, Ar-H), 7.60 (d, 2H, 8.4Hz, Ar-H), 7.64 (d, 1H, *J* = 8.5Hz, ArH), 8.05 (d, 1H, *J* = 6.3Hz, Ar-H). MS (m/z): 632 ($[M+H]^+$.

N-((7-((5-(4-Chlorophenyl)furan-2-yl)methylene)-6-(2,4-dichloro-5-fluorophenyl)-7H-[1,2,4]triazolo [3,4-b][1,3,4]thiadiazine-3-yl)methyl)-4-methylbenzenamine 3h: IR (KBr, cm^{-1}):3405 (N-H), 3005 (ArC-H), 2915 (C-H), 1638 (C=N), 1599 (C=C), 1095 (C-F), 840 (ArC-H def.), 735 (C-Cl). ^{1}H NMR (DMSO-d_6, δ ppm): 2.58 (s, 3H, CH_3), 4.92 (1H, s, NH), 5.83 (2H, s, N-CH_2), 6.71 (1H, s, exocyclic =CH), 7.01 (1H, d, *J* = 3.7 Hz, furan C_3H), 7.06 (d, 2H, Ar-H, 8.5Hz), 7.24 (d, 2H, Ar-H, 8.5Hz), 7.36 (1H, d, *J* = 3.7 Hz, furan C_4H), 7.39 (d, 2H, Ar-H, 8.5Hz), 7.50 (d, 2H, Ar-H, 8.5Hz), 7.66 (d, 1H, *J* = 8.5 Hz, ArH), 8.02 (d, 1H, *J* = 6.3 Hz, Ar-H). MS (m/z): 612 ($[M+H]^+$.

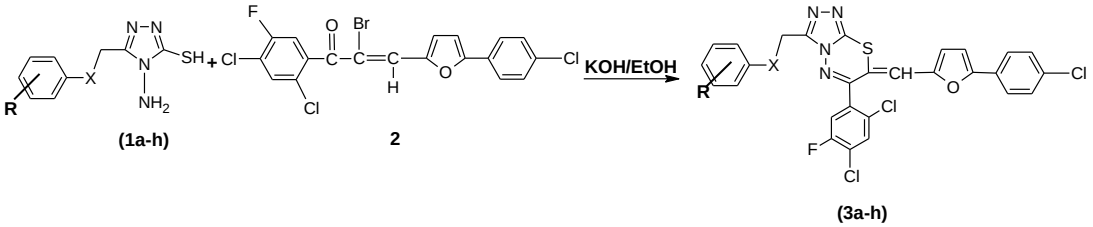
Antibacterial assay: Newly synthesized compounds were screened for their *in vitro* antibacterial activity against *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia Coli* and *Bacillus Subtilis* bacterial strains by serial dilution method [24]. The antibacterial activity of each compound was assessed by determining their Minimum Inhibitory Concentrations (MIC). 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. Nitrofurazone (furacin) was used as a standard drug for comparison and solvent control was kept.

Anticancer screening assay: Two of the newly synthesized compounds were acquired by the Drug Synthesis and Chemistry Branch, National Institute of Cancer, Bethesda, Maryland, USA in order to screen them for their anticancer activities under the Drug Discovery Programme [25-27] in a primary three

cell line-one dose anticancer assay. The three cell lines used in the present investigation are NCI-H460 (Lung), MCF 7 (Breast) and SF 268 (CNS). In the current protocol, each cell line is inoculated on a pre-incubated microtiter plate. The test agents are added at a single concentration and the culture is incubated for 48 h. End point determination are made with sulforhodamine B, a protein binding dye. Results for each test agents are reported as the percent growth of the treated cells when compared with the untreated control cells. Compounds which reduce the growth of any one of the cell lines to 32% or less (negative numbers indicate cell kill) are considered as active.

RESULTS AND DISCUSSION

The target compounds (**3a-h)** were synthesized as illustrated in **Scheme 1**. The reaction of various 4-amino-5-((aryloxy/arylamino)methyl)-*4H*-[1,2,4]-triazole-3-thiol(**1a-h**)with1-(2,4-dichloro-5-fluorophenyl -3-arylfuryl-2-bromo-2-propen-1-ones **2** in the presence of potassium hydroxide afforded the title compounds 7-arylfurylidene-6-(2,4-dichlorophenyl)-3-(aryloxymethyl/anilinomethyl)-*7H*-[1,2,4]-triazolo [3,4,*b*][1,3,4]thiadiazine (**3a-h**) in good yield. The starting material **1a-h** was prepared according to the literature procedure **[20].** The characterization data of the newly synthesized compounds are given in **table 1**.



X= O; R= 2-Cl, 4-Cl, 2,4-Cl_2, 4-Cl-3-Me, 3,4-Me_2 X= NH; R= H, 4-Me, 4-Cl.

Scheme 1. Synthesis of triazolothiadiazines derivatives (**3a-h**)

Table 1. Characterization data of 2-substituted-7-arylfurfuylidene-6-(2,4-dichloro-5-fluorophenyl)-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazines (**3a-h)**

Comp.	R	R^1	X	M.P. (^{0}C)	Mol. formula	Yield (%)	Elemental analysis % found (calculated) C	H	N
3a	2-Cl	4-Cl	O	148-50	$C_{28}H_{15}Cl_4FN_4O_2S$	68	53.02 (53.16)	2.32 (2.37)	8.79 (8.86)
3b	4-Cl	4-Cl	O	128-30	$C_{28}H_{15}Cl_4FN_4O_2S$	72	52.98 (53.16)	2.42 (2.37)	8.81 (8.86)
3c	2,4-Cl_2	4-Cl	O	210-12	$C_{28}H_{14}Cl_5FN_4O_2S$	65	50.30 (50.41)	2.03 (2.10)	8.44 (8.40)
3d	4-Cl-3-CH_3	4-Cl	O	148-50	$C_{29}H_{17}Cl_4FN_4O_2S$	69	53.70 (5387)	2.59 (2.63)	8.72 (8.67)
3e	3,4-$(CH_3)_2$	4-Cl	O	178-80	$C_{30}H_{20}Cl_3FN_4O_2S$	73	57.45 (57.50)	3.16 (3.20)	8.85 (8.91)
3f	H	4-Cl	NH	180-82	$C_{28}H_{17}Cl_3FN_5OS$	73	56.20 (56.27)	2.90 (2.85)	11.70 (11.74)
3g	4-CH_3	4-Cl	NH	224-25	$C_{29}H_{19}Cl_3FN_5OS$	69	56.86 (56.94)	3.07 (3.11)	11.40 (11.47)
3h	4-Cl	4-Cl	NH	184-86	$C_{28}H_{16}Cl_4FN_5OS$	75	53.08 (53.01)	2.49 (2.54)	11.14 (11.09)

The structures of newly synthesized compounds were established on the basis of elemental analyses, IR, ^{1}H NMR, ^{13}C NMR and Mass spectral data. The formation of these compounds (**3**) was confirmed by

recording their IR, ^{1}H NMR and mass spectra. The absence of characteristic absorption bands due to NH_2 (3237 cm^{-1}), SH (2592 cm^{-1}) and CO (1682 cm^{-1}) groups of the starting materials in the IR spectra of compounds **3** confirmed the formation of condensed products with cyclic structures. The IR spectrum of compounds **3a** showed the characteristic aromatic and alkyl stretching vibrations at 3010 and 2920 cm^{-1} respectively. The bands observed at 1630 and 1590 cm^{-1} were assigned to C=N and C=C stretching vibrations. Medium absorptions at 1090 and 730 cm^{-1} were due to C-F and C-Cl stretching. The IR spectrum of the compound **3a** showed the characteristic absorption bands at 3400 (NH), 3020 (ArC-H), 2912 (C–H), 1620 (C=N), 1593 (C=C), 1560 (NO_2), 1350 (NO_2), 1088 (C–F) and 752 cm^{-1} (C–Cl). The 300 MHz ^{1}H NMR spectrum of compound **3a** showed a singlet at δ 5.40 for its OCH_2 protons. The exocyclic vinylic protons resonated as a singlet at δ 6.51. Two protons of the furan ring resonated as two doublets at δ 6.92 (J = 3.7 Hz) and δ 7.36 (J = 3.7 Hz) respectively. The signals due to aromatic protons of 2,4-dichloro-5-fluorophenyl ring appeared as two doublets at δ 7.63 (J = 8.5 Hz) and δ 8.07 (J = 6.5 Hz) respectively. The difference in the coupling constants could be attributed to the coupling of fluorine with ortho and meta protons of dichlorofluoro substituted phenyl ring. The ^{1}H NMR spectrum of compound **3g** showed a singlet at δ 2.58 integrating for three protons of the methyl group. The NH protons appeared as a singlet at δ 4.92. The anilinomethyl protons resonated as a singlet at δ 5.83. The exocyclic vinylic proton resonated as a singlet at δ 6.71. The two protons of the furan ring resonated as two doublets at δ 7.01 and 7.32 respectively each with a J value of 3.7 Hz. The aromatic protons of 2,4-dichloro-5-fluorophenyl ring appeared as two doublets at δ 7.65 (J = 6.3 Hz) and δ 8.0 (J = 8.5 Hz) respectively. The signals due to remaining eight aromatic protons appeared as multiplets in the region of δ 7.34 and 8.05.

The FAB mass spectrum of compound **3a** showed the molecular ion peak at m/z 631 $(M + H)^+$ in conformity with the molecular formula $C_{28}H_{15}Cl_4FN_4O_2S$. A peak at m/z 504 was due to a cation formed by the loss of 2-chlorophenoxy radical from the molecular ion. The peaks at m/z 189 and 163 could be attributed to the (2,4-dichloro-5-fluorobenzonitrile radical cation and 2,4-dichloro-5-fluorophenyl cation respectively. Similarly, mass spectrum of compound **3s** showed the molecular ion peak at m/z 678 consistent with its molecular formula $C_{29}H_{16}Cl_5FN_4O_2S$. A peak at m/z 537 was attributed to the formation of a cation by the loss of 4-chloro-3-methylphenoxy radical from the molecular ion. A peak at m/z 155 was due to the formation of 4-chloro-3-methylphenoxymethyl cation. The peak at m/z 189/191 was attributed to the formation of 2,4-dichloro-5-fluorobenzonitrile ion-radical during fragmentation.

Antibacterial activity: The investigation of antibacterial screening data revealed that all the tested compounds **3a-h** showed moderate to very good inhibition in DMF. The minimum inhibitory concentrations (MIC values) of the compounds 3a-h are given in **table 2.**

Table 2: Antibacterial activity of triazolothiadiazines (MIC, μg mL^{-1})

Comp.	*S. aureus*	*P. aeruginosa*	*E. coli*	*B. subtilis*
3a	12.5	6.25	12.5	6.25
3b	6.25	12.5	12.5	6.25
3c	6.25	6.25	6.25	6.25
3d	12.5	12.5	6.25	12.5
3e	25.0	12.5	6.25	12.5
3f	12.5	6.25	12.5	12.5
3g	6.25	12.5	12.5	6.25
3h	6.25	6.25	6.25	6.25
Ampicillin	3.125	6.25	6.25	3.125

In the first series of compounds **3a-e** derived from substituted phenolic moiety **1a-e**, variation was introduced in the aryl furfural moiety with varying substitutions. Compound **3a** with chloro at 2nd position exhibited good activity against gram +ve strains with MIC value of 6.25μg mL^{-1} which is equipotent to the standard drug ampicillin, but moderate activity against gram +ve strains with MIC 12.5 μg mL^{-1}. When chloro group was shifted to the 3rd position, activity was completely get reversed in **3b**. It showed very good activity against gram positive strains with MIC 6.25 μg mL^{-1} and 12.5v against gram –ve bacterial strains. In **3c**, introduction of chloro at 2,4 position drastically enhances the antibacterial activity. It exhibited remarkable activity against all the strains with MIC 6.25 μg mL^{-1}. Further, compound **3d** in which chloro was at 4th position and an electron releasing methyl was at 3rd position completely decreased the activity. Moreover, compound **3e** which has electron releasing methyl at 3,4position completely resulted poor activity with MIC 25 μg mL^{-1}. From the second series of compounds **3f-h** derived from aniline moiety **1c**, showed moderate to good antibacterial activity. Compound **3h** with chloro at 4th position of aryl furfural exhibited very good activity against all the strains with MIC 6.25μg mL^{-1}. This compound **3h** and compound **3c** from the first series exhibited same degree of activity. Moreover, un-substituted compound **3f** showed moderate activity against all the strains. But, compound **3g** with electron releasing methyl at 4th position exhibited good activity against gram +ve atrains but oderate against gram-ve strains.

Anticancer activity: In the preliminary screening program the compounds 3d and 3h have been found to be active. Results of this study are given in table 3. The active compounds 3d and 3h are passed on for extensive evaluation in the full panel of 60 cell lines over a five- log dose range, they showed variable antitumor property against most of the tested subpanel tumor cell lines at GI_{50} level. The GI_{50} values of these two compounds are given in table 4.

Table 3: Preliminary in vitro anticancer screening data of triazolothiadiazines 3d and 3h.

Compound	NCS Code	Sample concentration 10^{-4}M	Growth Percentage[a]			Activity[b]
			Lung (NCI-H 460)	Breast (MCF-7)	CNS (SF-268)	
3d	719149	1.00	39	31	53	Active
3h	719148	1.00	43	30	41	Active

[a]Percent cell growth reduction following 48-h incubation with test compounds (optical density, sulforhodamine procedure).
[b]Active when growth percentage is < 32% for any of the three cell lines.

Table 4: GI_{50}(μM) values for compounds (**3d** and **3h**)

Cell line	GI_{50} (□ M)	
	3d	**3h**
Leukemia		
CCRF-CEM	>50.12	67.61
HL-60(TB)	>50.12	>100
K-562	26.92	12.02
MMOLT-4	>50.12	3.31
RPMI-8226	14.13	23.43
SR	22.91	29.51
Non-small cell lung cancer		
A 549/A TCC	>50.12	35.48
EKVX	16.22	7.59
HOP-62	>50.12	21.88
HOP-92	--	--
NCI-H226	36.31	26.30
NCI-H23	26.30	81.38
NCI-H322M	>50.12	33.88
NCI-H460	29.51	64.57
NCI-H522	28.84	38.02

Colon cancer		
COLO 205	10.47	14.45
HCC-2998	19.06	57.54
HCT-116	47.86	63.10
HCT-15	33.88	>100
HT29	21.38	33.11
KM12	41.69	>100
SW-620	3.11	>100
CNS cancer		
SF-268	>50.12	53.70
SF-295	36.31	22.30
SF-539	--	--
SNB-19	>50.12	43.65
SNB-75	--	--
U251	>50.12	18.62
Melanoma		
LOX IMVI	31.62	22.91
MALME-3M	3.89	19.50
M14	>50.12	42.66
SK-MEL-2	>50.12	22.39
SK-MEL-28	25.12	67.61
UACC-62	41.69	26.30
UACC-257	>50.12	>100
UACC-62	18.20	13.49
Ovarian cancer		
IGROV1	12.02	17.78
OVCAR-3	30.20	29.51
OVCAR-4	--	--
OVCAR-5	>50.12	18.62
OVCAR-8	>50.12	51.29
SK-OV-3	--	--
Renal cancer		
786-0	>50.12	32.36
A 498	24.55	17.38
ACHN	>50.12	34.67
CAKI-1	39.81	23.43
RXF 393	>50.12	13.49
SN12C	31.62	33.11
TK-10	40.74	17.38
UO-31	48.98	11.75
Prostate cancer		
PC-3	14.43	14.79
DU-145	>50.12	64.57
Breast cancer		
MCF7	20.42	38.90
NCI/ADR-RES	12.02	6.46
MDA-MB-231/ATCC	12.88	10.23
HS 578T	>50.12	48.77
MDA-MB-435	19.50	69.18
MDA-N	21.38	>100
BT-549	>50.12	>100
T-47D	7.94	41.69

Compounds **3d** and **3h** showed good antiproliferative activity against some of the cell lines. The cell lines show activity with GI_{50} values <10 μM for compound **3d** are SW-620, MALME-3M and T-47D. On the other hand, compound **3h** showed GI_{50} values <10 μM for MMOLT-4, EKVX and NCI/ADR-RES cell lines. The compound **3d** (R=4-Cl, 3-CH_3, R^1=4-Cl) showed the highest activity against SW-620 cell line (GI_{50}, 3.11μM), whereas compound **3h** (R=4-Cl, R^1=4-Cl) showed the highest activity against Leukemia MMOLT–4 cell line (GI_{50}, 3.31 μM). Further compound **3d** showed very good activity against Melanoma MALME-3M cell line (GI_{50}, 3.89μM) and Breast cancer T-47D cell line (GI_{50}, 7.94μM). Also compound

3h exhibited very good anticancer activity against Non-small cell lung cancer EKVX (GI_{50}, 7.59μM) and against Breast cancer NCI/ADR-RES (GI_{50}, 6.46μM).

However here it is not possible to explain structure-activity relationship in these two compounds, but it appears that presence of 4-chloro-3-methyl-aryloxy methyl, 2-chloroaryloxy methyl at C3 position and 4-chloro arylfurfurylidene at C7 substitution contribute their significance imparting anticancer property. Also in our previously reported paper [20], compound containing 4-chloro-3-methyl-aryloxy methyl at C3 position and 4-chloro benzylidene at C7 substitution showed significant anticancer activity.

APPLICATIONS

The target compounds are synthesized in good yield and a few compounds are emerged as potent antibacterial agents which could be further screened for in vivo studies to know its efficacy. Also two anticancer compounds could be used in animal model of anticancer study to evaluate its potency.

CONCLUSIONS

This research paper describes the successful synthesis of new series of 7-arylfurylidene-6-(2,4-dichlorophenyl)-3-(aryloxymethyl/anilinomethyl)-*7H*-[1,2,4]-triazolo[3,4-*b*][1,3,4]thiadiazine and *in-vitro* antibacterial and anticancer evaluation. Antibacterial study reveals that the all the tested compounds exhibited better activity against all the bacterial strains. In particular, compounds **3c** and **3h**, exhibit excellent antibacterial activity at MIC 6.25. It was well pronounced from the study that the presence of halogenated moiety is essential for excellent antibacterial activity. In the case of anticancer activity, only two compounds **3d** and **3h** were emerged as potent. These two compounds were selected on a random basis; therefore we are quite unable to discuss the structure activity relationship, as it is a preliminary screening. From our earlier studies we found that 2,4-dichloro-5-fluorophenyl moiety was a good synthon for the preparation of newer heterocycles. Thus, we conclude that there is better scope for triazolothiadiazines class of compounds for further development as antibacterial and anticancer agents.

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