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Short communication

Synthesis and studies on some new fluorine containing triazolothiadiazines as possible antibacterial, antifungal and anticancer agents

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Abstract

Synthesis of a series of 7-arylidene-6-(2,4-dichlorophenyl)-3-aryloxymethyl/anilinomethyl-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazines (**3**) by the condensation of 3-aryl-1-(2,4-dichloro-5-fluorophenyl)-2-bromo-propen-1-one (**1**) and 4-amino-5-mercapto-3-aryloxymethyl/anilinomethyl-1,2,4-triazoles (**2**) is described. The newly synthesized compounds were characterized by elemental analysis IR, ^{1}H NMR and mass spectral data. These compounds were tested for their antimicrobial activities against *Escherichia coli*, *Staphylococcus aureus* (Smith), *Psuedomonas aeruginosa* (Gessard), *Bacillus subtilis* and *Candida albicans*. Some of the newly synthesized compounds were also screened for their anticancer activity. Among them compounds **3m**, **3o**, **3q** showed in vitro anticancer activity.

Keywords: 1,2,4-Triazoles; 1,3,4-Thiadiazines; Antibacterial agents; Antifungal agents; Anticancer screening

1. Introduction

1,2,4-Triazoles and their derivatives play important role in medicinal, agricultural and industrial fields [1–4]. N-bridged heterocyclic derivatives derived from 1,2,4-triazoles show varied biological activities [5]. Recently, fluorinated heterocycles have attracted attention due to the ability of fluorine to act as polar hydrogen or hydroxyl mimic. Therefore substitution of hydrogen by fluorine has been a strategy in designing molecules for biological activity studies [6,7]. In continuation of our search on the synthesis of biologically active fluorine containing N-bridged heterocycles [8,9] and in an attempt to significantly improve antibacterial and anticancer activities of triazlothiadiazine (general structure 1) we considered substitution at C3 by aryloxymethyl and anilinomethyl moieties. It was hoped that these compounds in addition to retaining anticancer properties of previously reported 3-akyl/aryl-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazines [8], would benefit from the incorporation of aryloxy and anilinomethyl group. A similar strategy on the triazolothiadiazine template to produce antibacterials comparable or superior to nitrofurazone; actually, we found that replacement of alkyl and aryl group by aryloxy and anilinomethyl substituents was not advantageous. In the case antitumor activity in these template three compounds with 2-chlorophenoxymethyl, 4-chloro-3-methyl-phenoxymethyl and 4-chloro-anilinomethyl as substituent at C3 found to be active. Moreover, screening of these triazolothiadiazines was expanded to include antifungal activity.

2. Results and discussion

2.1. Chemistry

The synthesis of reported triazolothiadiazines **3a**–**3b**[1] was carried out by the condensation of 3-aryl-1-(2,4-dichloro-5-fluorophenyl)-2-bromo-2-propen-1-one (**1**) with 4-amono-5-

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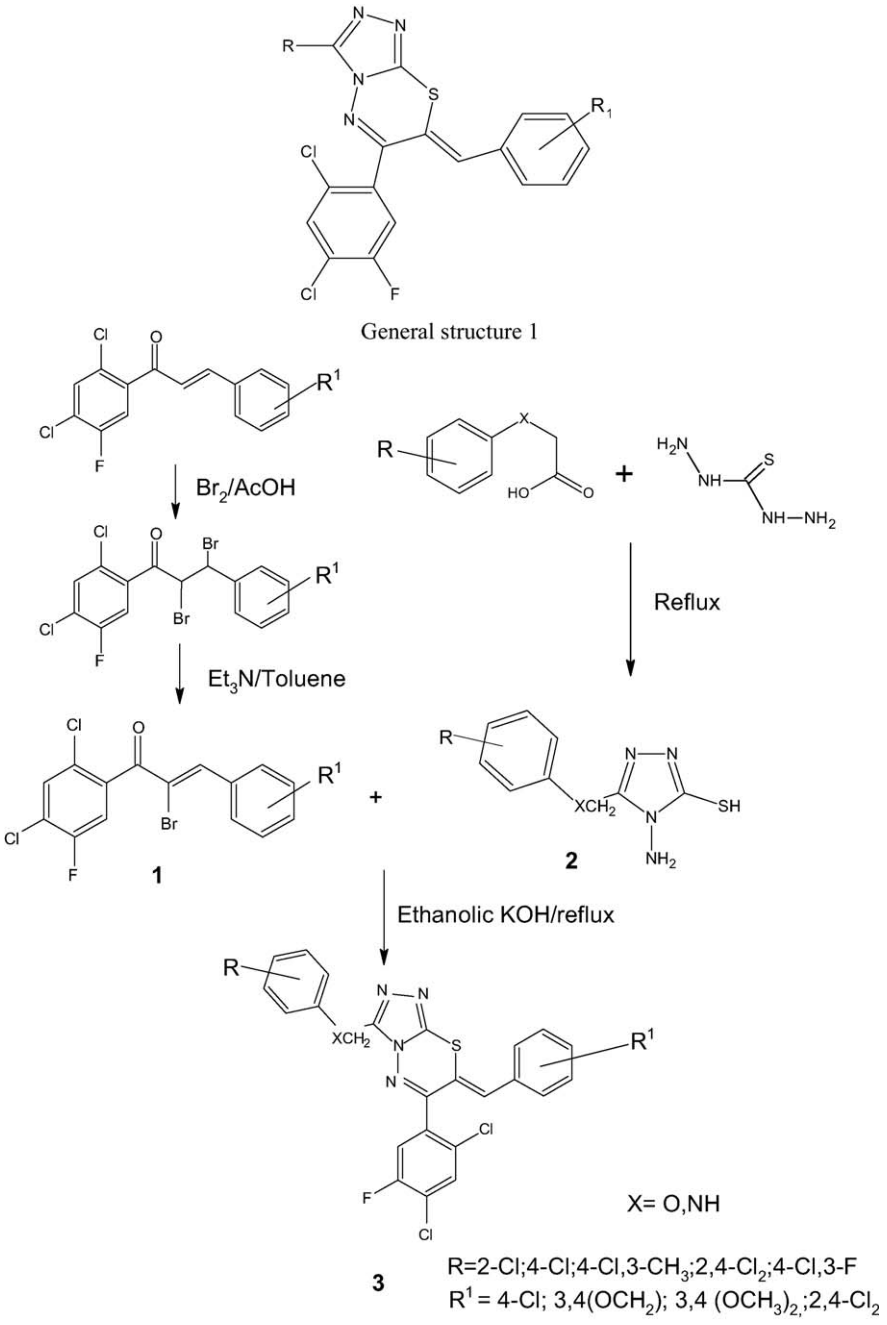
mercapto-5-aryloxymethyl/anilinomethyl-1,2,4-triazoles (**2**) in presence of alcoholic potassium hydroxide (Scheme 1). The structure of newly synthesized compounds was confirmed by recording the IR, ^{1}H NMR and mass spectra. Characterization data of triazolothiadiazines are given in Table 1. IR spectrum of **3i** showed absorption bands at 3034, 2834, 1585, 1081 and 728 cm^{-1} due to CH, C=N, C–F, and C–Cl groups, respectively. The absence of the absorption bands corresponding to –NH_2, –SH and C=O stretching frequency of the reactants clearly confirmed the formation of triazolothiadiazines **3a**–**3b**[1]. The IR spectra of other compounds of the series showed similar absorption bands and the data are listed in Table 1. The ^{1}H NMR spectrum of **3n** showed a singlet at δ 6.65 corresponding to exocyclic vinylic proton. The signals due to six protons of 3,4-dimethoxy group appeared as two closely packed singlets at 3.91 and 3.94, respectively. The six aromatic protons of 3,4-dimethoxyphenyl and 3-chloro 4-fluoro phenyl ring resonated as multiplets in the region δ 6.64–7.08. The aromatic protons of the 2,4-dichloro-5-fluorophenyl ring resonated as two doublets at δ 7.33 (J = 8.5 Hz, ortho H–F coupling) and δ 7.66 (J = 6 Hz, meta H–F coupling) integrating for one proton, respectively, this data confirmed the formation of compound **3n**. The ^{1}H NMR spectra of some of the compounds were recorded and their spectral data are given in Table 1. Mass spectral data of the newly synthesized compounds were recorded are also given in Table 1. Almost all the compounds



Scheme 1. Preparation of 7-arylidene-6-(2,4-dichloro5-fluorophenyl)-3-aryloxymethyl/anilinomethyl-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazines (**3**).

Table 1
Characterization data of 7-arylidene-6-(4-dichloro-5-fluorophenyl)-3-substituted-1,2,4-triazolo [3,4-b]-1,3,4-thiadiazine (**3**)

Compound numbers	R	R^1	X	M.p. (°C)	Yield (%)	Molecular formula	Analysis (%) Found (Calculated)		
							C	H	N
3a	2-Cl	3,4-(OCH_2O)	O	246–248	74	$C_{25}H_{14}Cl_3FN_4O_3S$	52.011 (52.12)	2.52 (2.43)	9.61 (9.73)
3b	4-Cl	3,4-(OCH_2O)	O	296–298	78	$C_{25}H_{14}Cl_3FN_4O_3S$	51.94 (52.12)	2.30 (2.43)	9.82 (9.73)
3c	4-Cl, 3-CH_3	3,4-(OCH_2O)	O	168–169	71	$C_{26}H_{16}Cl_3FN_4O_3S$	52.71 (52.96)	2.69 (2.71)	9.64 (9.49)
3d	2,4-Cl_2	3,4-(OCH_2O)	O	185–187	68	$C_{25}H_{13}Cl_4FN_4O_3S$	48.91 (49.18)	2.04 (2.13)	9.02 (9.18)
3e	H	3,4-(OCH_2O)	NH	180–181	76	$C_{25}H_{16}Cl_2FN_5O_2S$	55.21 (55.55)	2.84 (2.86)	12.64 (12.96)
3f	4-Cl	3,4-(OCH_2O)	NH	215–117	73	$C_{25}H_{15}Cl_3FN_5O_2S$	52.06 (52.21)	2.58 (2.61)	12.06 (12.18)
3g	3-Cl 4-F	3,4-(OCH_2O)	NH	158–160	81	$C_{25}H_{14}Cl_3F_2N_5O_2S$	50.34 (50.63)	2.28 (2.36)	11.64 (11.8)
3h	2-Cl	3,4-(OCH_3)$_2$	O	198–200	76	$C_{26}H_{18}Cl_3FN_4O_3S$	52.56 (52.74)	2.95 (3.04)	9.23 (9.46)
3i	4-Cl	3,4-(OCH_3)$_2$	O	160–162	74	$C_{26}H_{18}Cl_3FN_4O_3S$	52.42 (52.74)	2.91 (3.04)	9.28 (9.46)
3j	4-Cl, 3-CH_3	3,4-(OCH_3)$_2$	O	165–167	78	$C_{27}H_{20}Cl_3FN_4O_3S$	53.24 (53.51)	3.23 (3.30)	7.74 (7.92)
3k	2,4-Cl_2	3,4-(OCH_3)$_2$	O	184–186	71	$C_{26}H_{17}Cl_4FN_4O_3S$	49.67 (49.84)	2.59 (2.71)	8.78 (8.94)
3l	H	3,4-(OCH_3)$_2$	NH	173–175	73	$C_{26}H_{20}Cl_2FN_5O_2S$	55.88 (56.11)	3.61 (3.59)	12.34 (12.59)
3m	4-Cl	3,4-(OCH_3)$_2$	NH	201–203	79	$C_{26}H_{19}Cl_3FN_5O_2S$	52.57 (52.83)	3.16 (3.21)	11.62 (11.85)
3n	3-Cl, 4-F	3,4-(OCH_3)$_2$	NH	174–176	75	$C_{26}H_{18}Cl_2F_2N_5O_2S$	51.04 (51.27)	2.90 (2.95)	11.36 (11.50)
3o	2-Cl	4-Cl	O	168–170	68	$C_{24}H_{13}Cl_4FN_4OS$	50.61 (50.88)	2.26 (2.29)	9.71 (9.89)
3p	4-Cl	4-Cl	O	178–180	77	$C_{24}H_{13}Cl_4FN_4OS$	50.58 (50.88)	2.24 (2.29)	9.74 (9.89)
3q	4-Cl, 3-CH_3	4-Cl	O	180–182	71	$C_{25}H_{15}Cl_4FN_4OS$	51.48 (51.72)	2.61 (2.58)	9.48 (9.65)
3r	2,4-Cl_2	4-Cl	O	168–170	76	$C_{24}H_{12}Cl_5FN_4OS$	47.54 (47.96)	1.91 1.99	9.18 (9.32)
3s	H	4-Cl	NH	182–184	80	$C_{24}H_{15}Cl_3FN_5S$	54.07 (54.11)	2.80 (2.82)	13.04 (13.19)
3t	4-Cl	4-Cl	NH	226–228	82	$C_{24}H_{14}Cl4FN_5S$	50.63 (50.97)	2.41 (2.47)	12.28 (12.39)
3u	3-Cl, 4-F	4-Cl	NH	196–198	76	$C_{24}H_{13}Cl_5F_2N_5S$	49.08 (49.39)	2.26 (2.23)	12.09 (12.00)
3v	2-Cl	2,4-Cl_2	O	196–198	70	$C_{24}H_{13}Cl5FN_4OS$	47.62 (47.96)	1.95 (1.99)	9.24 (9.32)
3w	4-Cl	2,4-Cl_2	O	175–175	77	$C_{24}H_{12}Cl_5FN_4OS$	47.71 (47.96)	1.93 (1.99)	9.16 (9.11)
3x	4-Cl, 3-CH_3	2,4-Cl_2	O	96–98	78	$C_{25}H_{14}Cl_5FN_4OS$	48.51 (48.82)	2.22 (2.29)	9.16 (9.11)
3y	2,4-Cl_2	2,4-Cl_2	O	166–168	81	$C_{24}H_{11}Cl_6FN_4OS$	45.12 (45.35)	1.71 (1.73)	8.73 (8.81)
3z	H	2,4-Cl_2	NH	178–180	80	$C_{24}H_{14}Cl_4FN_5S$	54.07 (54.11)	2.80 (2.82)	13.04 (13.19)
3a[1]	4-Cl	2,4-Cl_2	NH	198–200	82	$C_{24}H_{14}Cl_4FN_5S$	50.63 (50.97)	2.41 (2.47)	12.28 (12.39)
3b[1]	3-Cl, 4-F	2,4-Cl_2	NH	93–95	76	$C_{24}H_{13}Cl_5F_2N_5S$	49.08 (49.39)	2.26 (2.23)	12.09 (12.00)

3i: IR (KBr disc) γ_{max} (cm^{-1}): 3034 (C–H), 2834 (C–O), 1590 (C=N), 1081 (C–F), 728 (C–Cl); **3j**: IR (KBr disc) γ_{max} (cm^{-1}): 2965 (C–H), 1597 (C–N), 1514 (C=C), 1086 (C–F), 731 (C–Cl); **3k**: IR (KBr disc) γ_{max} (cm^{-1}): 3945 (C–H), 1605 (C–N), 1512 (C=C), 1076 (C–F), 731 (C–Cl); **3c**: ^{1}H NMR 400 MHz ($CDCl_3$): δ, 7.29 (d, 1H, Ar–H, $J_{H-F\ ortho}$ = 8.1 Hz), 7.49 (m, 2H, Ar–H), 7.61 (d, 1H, Ar–H, $J_{H-F\ meta}$ = 6.3 Hz), 7.73 (s, 1H, =CH–), 7.9 (m, 2H, Ar–H); 3d: ^{1}H NMR 400 MHz ($CDCl_3$): δ, 7.27 (d, 1H, Ar–H, J = 8 Hz), 7.37 (dd, 1H, Ar–H, J = 2 Hz, J = 2 Hz), 7.48 (d, 1H, Ar–H, J = 2.1 Hz), 7.57 (d, 1H, Ar–H, $J_{H-F\ meta}$ = 6.3 Hz), 7.86, (s, 1H, CH, 8.02 (d, 1H, Ar–H, $J_{H-F\ ortho}$ = 8.5 Hz); **3f**: ^{1}H NMR 400 MHz ($CDCl_3$): δ, 3.92 (s, 1H, NH), 4.6 (s, 2H, –CH_2–), 6.67 (s, 2H, –OCH_2O–), 6.71 (s, 1H, –CH=), 7.11–7.14 (m, 7H, Ar–H), 7.36 (d, 1H, Ar–H, $J_{H-F\ ortho}$ = 8.3 Hz), 7.63 (d, 1H, Ar–H, $J_{H-F\ meta}$ = 6.5 Hz); **3o**: ^{1}H NMR 400 MHz ($CDCl_3$): δ, 5.43 (s, 2H, –OCH_2–), 6.96 (s, 1H, –CH=), 7.59 (d, 1H Ar–H, $J_{H-F\ meta}$ = 6.5 Hz), 6.93–7.53 (m, 9H, Ar–H); **3n**: ^{1}H NMR 300 MHz ($CDCl_3$ DMSO-d_6): δ, 3.91 (s, 1H, OCH_3), 3.94 (s, 3H, –OCH_3), 4.4 (t, 1H, NH), 4.56 (d, 2H, –CH_2–), 6.65 (s, 1H, –CH=), 7.32 (d, 1H, Ar–H, $J_{H-F\ ortho}$ = 8.5 Hz), 7.65 (d, 1H, Ar–H, $J_{H-F\ meta}$ = 6.5 Hz), 6.57–7.08 (m, 6H, Ar–H); **3t**: ^{1}H NMR 300 MHz ($CDCl_3$): δ, 4.36 (s, 1H, –NH–), 4.56 (s, 2H, –CH_2–), 6.69 (s, 1H, –CH=). 6.56–7.44 (m, 2H, Ar–H), 7.66 (d, 1H, Ar–H $J_{H-F\ meta}$ = 6.5 Hz); **3w**: ^{1}H NMR 300 MHz ($CDCl_3$): δ, 5.29 (s, 2H, –OCH_2–), 6.85 (s, 1H, –CH=), 6.97–7.53 (m, 8H, Ar–H), 7.63 (d, 1H, Ar–H, $J_{H-F\ meta}$ = 8.6 Hz); **3x**: ^{1} H NMR 300 MHz ($CDCl_3$): δ, 2.32 (s, 3H, –CH_3), 5.26 (s, 2H, –OCH_2–), 6.71 (s, 1H, –CH=), 6.79–7.71 (m, 8H, Ar–H); **3y**: ^{1}H NMR 300 MHz ($CDCl_3$): δ, 5.30 (s, 2H, –OCH_2–), 6.87 (s, 1H, –CH=), 6.99–7.62 (m, 7H, Ar–H), 7.87 (d, 1H, Ar–H, $J_{H-F\ ortho}$ = 8.6 Hz); **3a**: *m/z* 574 (80%, M^+), 407 (80%, M^+-2-chloroaryloxy methyl nitrile radical), 189 (100%, 2,4-dichloro-5-fluorobenzonitrile), 163 (4%, 2,4-dichloro-5-flurophenyl cation) 121 (60% 3,4-methylenedioxyphenyl cation); **3b**: *m/z* 574 (70%, M^+), 407 (80%, M^+-4-chloroaryloxy methyl nitrile radical), 189 (100%, 2,4-dichloro-5-fluorobenzonitrile), 163 (4%, 2,4-dichloro-5-flurophenyl cation) 121(50% 3,4-methylenedioxyphenyl cation); **3c**: *m/z* 588 (90%, M^+), 407 (80%, M^+-4-chloro-3-methyl-aryloxy methyl nitrile radical), 189 (100%, 2,4-dichloro-5-fluorobenzonitrile), 163 (80%, 2,4-dichloro-5-flurophenyl cation) 121 (60% 3,4-methylenedioxyphenyl cation); **3d**: *m/z* 608 (50%, M^+), 407 (80%, M^+-2,4-dichloroaryloxy methyl nitrile radical), 189 (100%, 2,4-dichloro-5-fluorobenzonitrile), 163 (44%, 2,4-dichloro-5-flurophenyl cation) 121 (50% 3,4-methylenedioxyphenyl cation); **3e**: *m/z* 539 (20%, M^+), 407 (90%, M^+-anilinomethyl nitrile radical), 189 (10%, 2,4-dichloro-5-fluorobenzonitrile), 163 (100%, 2,4-dichloro-5-flurophenyl cation) 121 (60% 3,4-methylenedioxyphenyl cation); **3f**: *m/z* 573 (30%, M^+), 407 (80%, M^+-4-chloroanilinomethyl nitrile radical), 189 (70%, 2,4-dichloro-5-fluorobenzonitrile), 163 (100%, 2,4-dichloro-5-flurophenyl cation) 121 (60% 3,4-methylenedioxyphenyl cation); **3g**: *m/z* 591 (30%, M^+), 407 (90%, M^+-3-chloro-4-fluoro-anilinomethyl nitrile radical), 189 (25%, 2,4-dichloro-5-fluorobenzonitrile), 163 (100%, 2,4-dichloro-5-flurophenyl cation) 121 (60% 3,4-methylenedioxyphenyl cation); **3h**: *m/z* 590 (30%, M^+), 423 (80%, M^+-2-chloroaryloxy methyl nitrile radical), 189 (100%, 2,4-dichloro-5-fluorobenzonitrile), 163 (14%, 2,4-dichloro-5-flurophenyl cation) 137 (60% 3,4-dimethoxyphenyl cation); **3i**: *m/z* 590 (40%, M^+), 423 (80%, M^+-4-chloroaryloxy methyl nitrile radical), 189 (100%, 2,4-dichloro-5-fluorobenzonitrile), 163 (24%, 2,4-dichloro-5-flurophenyl cation) 137 (60% 3,4-dimethoxyphenyl cation); **3j**: *m/z* 604 (50%, M^+), 423 (60%, M^+-4-chloro-3-methyl-aryloxymethyl nitrile radical), 189 (100%, 2,4-dichloro-5-fluorobenzonitrile), 163 (4%, 2,4-dichloro-5-flurophenyl cation) 137 (80% 3,4-dimethoxyphenyl cation); **3k**: *m/z* 628 (70%, M^+), 423 (80%, M^+-2,4-dichloroaryloxymethyl nitrile radical), 189 (100%, 2,4-dichloro-5-fluorobenzonitrile), 163 (4%, 2,4-dichloro-5-flurophenyl cation) 137 (50% 3,4-dimethoxyphenyl cation); **3l**: *m/z* 555 (20%, M^+), 423 (90%, M^+-anilinomethyl nitrile radical), 189 (10%, 2,4-dichloro-5-fluorobenzonitrile), 163 (100%, 2,4-dichloro-5-flurophenyl cation) 137 (60% 3,4-dimethoxyphenyl cation); **3m**: *m/z* 589 (50%, M^+), 423 (70%, M^+-4-chloro-anilinomethyl nitrile radical), 189 (30%, 2,4-dichloro-5-fluorobenzonitrile), 163 (100%, 2,4-dichloro-5-flurophenyl cation) 137 (60% 3,4-dimethoxyphenyl cation); **3n**: *m/z* 607 (100%, M^+), 423 (80%, M^+-3-chloro-4-fluoro anilino radical), 444 (56%, M^+-2,4-dichloro-5-fluorophenyl radical), 189 (6%, 2,4-dichloro-5-fluorobenzonitrile), 163 (4%, 2,4-dichloro-5-flurophenyl cation); **3o**: *m/z* 564 (80%, M^+), 397 (80%, M^+-2-chloroaryloxy methyl nitrile radical), 189 (100%, 2,4-dichloro-5-fluorobenzonitrile), 163 (4%, 2,4-dichloro-5-flurophenyl cation) 111 (60% 4-chlorophenyl cation); **3p**: *m/z* 564 (80%, M^+), 397 (50%, M^+-4-chloroaryloxy methyl nitrile radical), 189 (100%, 2,4-dichloro-5-fluorobenzonitrile), 163 (4%, 2,4-dichloro-5-flurophenyl cation) 111 (60% 4-chlorophenyl cation); **3q**: *m/z* 578 (80%, M^+), 397 (80%, M^+-M^+-4-chloro-3-methyl-aryloxymethyl nitrile radical), 189 (100%, 2,4-dichloro-5-fluorobenzonitrile), 163 (4%, 2,4-dichloro-5-flurophenyl ca-

tion) 111 (70% 4-chlorophenyl cation); **3r**: *m/z* (M^+ is not observed), 397 (8%, M^+-2,4-$Cl_2C_6H_3OCH_2CN^+$) 189 (14%, 2,4-dichloro-5-fluorobenzonitrile), 163 (22%, 2,4-dichloro-5-flurophenyl cation) 111 (100% 4-chlorophenyl cation); **3s**: *m/z* 510 (20%, M^+), 397 (80%, M^+-anilinomethyl nitrile radical), 189 (100%, 2,4-dichloro-5-fluorobenzonitrile), 163 (4%, 2,4-dichloro-5-flurophenyl cation) 111 (60% 4-chlorophenyl cation); **3t**: *m/z* 544 (20%, M^+), 397 (80%, M^+-4-chloroanilinomethyl nitrile radical), 189 (100%, 2,4-dichloro-5-fluorobenzonitrile), 163 (4%, 2,4-dichloro-5-flurophenyl cation) 111 (60% 4-chlorophenyl cation) **3u**: *m/z* 562 (5%, M^+), 397 (70%, M^+-3-chloro-4-fluoro-anilinomethyl nitrile radical), 189 (100%, 2,4-dichloro-5-fluorobenzonitrile), 163 (4%, 2,4-dichloro-5-flurophenyl cation) 111 (60% 4-chlorophenyl cation) **3v**: *m/z* 599 (5%, M^+), 432 (90%, M^+-2-chloroaryloxy methyl nitrile radical), 189 (100%, 2,4-dichloro-5-fluorobenzonitrile), 163 (4%, 2,4-dichloro-5-flurophenyl cation) 145 (60% 2,4-dichlorphenyl cation); **3w**: *m/z* 599 (5%, M^+), 432 (90%, M^+-4-chloroaryloxy methyl nitrile radical), 189 (100%, 2,4-dichloro-5-fluorobenzonitrile), 163 (24%, 2,4-dichloro-5-flurophenyl cation) 145 (60% 2,4-dichlorphenyll cation); **3x**: *m/z* 613 (5%, M^+), 432 (90%, M^+-4-chloro-3-methyl-aryloxy methyl nitrile radical), 189 (100%, 2,4-dichloro-5-fluorobenzonitrile), 163 (34%, 2,4-dichloro-5-flurophenyl cation) 145 (60% 2,4-dichlorphenyll cation); **3y**: *m/z* 633 (5%, M^+), 432 (90%, M^+-2,4-dichloro-aryloxy methyl nitrile radical), 189 (100%, 2,4-dichloro-5-fluorobenzonitrile), 163 (4%, 2,4-dichloro-5-flurophenyl cation) 145 (60% 2,4-dichlorphenyll cation); **3z**: *m/z* 563 (20%, M^+), 432 (80%, M^+-anilinomethyl nitrile radical), 189 (100%, 2,4-dichloro-5-fluorobenzonitrile), 163 (4%, 2,4-dichloro-5-flurophenyl cation) 145 (50% 2,4-dichlorphenyll cation); **3a[1]**: *m/z* 597 (20%, M^+), 432 (80%, M^+-4-chloro-anilinomethyl nitrile radical), 189 (100%, 2,4-dichloro-5-fluorobenzonitrile), 163 (4%, 2,4-dichloro-5-flurophenyl cation) 145 (60% 2,4-dichlorphenyl cation); **3b[1]**: *m/z* 615 (20%, M^+), 432 (80%, M^+-3-chloro-4-fluoro-anilinomethyl nitrile radical), 189 (100%, 2,4-dichloro-5-fluorobenzonitrile), 163 (4%, 2,4-dichloro-5-flurophenyl cation) 145 (80% 2,4-dichlorphenyl cation).

showed molecular ion peaks revealing the stability of the compounds. The major peaks in the spectra appeared were due to the loss of aryloxy/anilinomethyl nitrile radical from the molecular ion, formation of 2,4-dichloro-5-fluoro benzonitrile radical ion and aryl cations.

2.2. Anticancer activity

In the present anticancer screening program, compounds **3m**, **3o**, **3q** possessed growth percentage to less than 32% against all the tested cell lines and were regarded as active compounds (Table 2). These three compounds **3m**, **3o** and **3q** were then passed on for evaluation in the full panel of 60 cell lines derived from seven cancer types namely, lung, colon, melanoma, renal, ovarian, CNS and leukemia. These compounds showed antiproliferative activity on the whole cell panel, although they did not prove cytotoxic or cytostatic at the maximum tested concentration (100 μM). The screening data are presented in Table 3. Compound **3o** [2-chloroaryloxy methyl at C3 and 4-chloro benzylidene at C7 substitution] showed highest activity with GI_{50} value < 10 μM against all tested 60 cell lines. Whereas compound **3q** [4-chloro-3-methyl-aryloxy methyl at C3 and 4-chloro benzylidene at C7 substitution] showed moderate activity GI_{50} value < 50 μM against all tested 60 cell lines though it showed highest activity GI_{50} value = 0.336 μM against leukemia MOLT-4 cell line among active compounds. Compound **3m** [4-chloro-anilinomethyl at C3 and 3,4-dimethoxy benzylidene at C7 substitution] appears to be least active with GI_{50} value > 100 μM for some of the tested cell lines. In the alkyl/aryl substituted triazolothiadiazine series compounds with methyl group at C3 and 3,4-methylenedioxy benzylidene at C7, methyl at C3 and 4-chloro benzylidene at C7, methyl at C3 and 3,4-dimethoxy benzylidene at C7 and phenyl group at C3 and 3,4-diomethoxy benzylidene at C7 were found to be active. With this information it is immature to comment on structure activity relationship. However, it appears to be the presence of 4-chloro benzylidene and 3,4-dimethoxy benzylidene at C7 may also contribute to their activity.

2.3. Antibacterial activity

The newly synthesized thiadiazines **3** were screened for their antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* (Smith), *Psuedomonas aeruginosa* (Gessard) and *Bacillus subtilis* bacterial strains by serial dilution method [11]. Almost all compounds exhibited comparable activity with standard nitrofurazone, but did not prove to be more active than the previously reported compounds in this template. Compound **3i**, 7-(3,4-dimethoxy benzylidene)-6-(2,4-dichloro-5-

Table 2
Preliminary in vitro anticancer screening a data of triazolothiadiazines

Compound numbers	NSC number	Sample concentration × 10^{-4} (M)[a]	Growth percentage[b]			Activity[c]
			Lung NCI-H 460	Breast MCF 7	CNS SF-268	
3a	713313	1.00	58	65	89	Inactive
3b	713314	1.00	83	89	44	Inactive
3f	713319	1.00	44	24	59	Inactive
3h	713315	1.00	47	49	73	Inactive
3i	713316	1.00	61	66	88	Inactive
3j	713317	1.00	66	58	79	Inactive
3m	713320	1.00	16	10	48	*Active*
3o	713310	1.00	–30	0	14	*Active*
3p	713311	1.00	70	67	93	Inactive
3q	713312	1.00	–30	–10	–14	*Active*
3t	713318	1.00	97	73	32	Inactive

[a] Fixed concentration assay (100 μM; standard NCI protocol).
[b] Percent cell growth reduction following 48-h incubation with test compounds (optical density, sulforhodamine procedure).
[c] Active when growth percentage is < 32% for any of the three cell lines.

Table 3
Sixty cell line in vitro anticancer screening (GI_{50}, μM) triazolothiadiazines **3m**, **3o** and **3q**

Panel/cell line	GI_{50} (μM)		
Leukemia	**3m**	**3o**	**3q**
CCRF-CEM	4.97	9.03	2.95
HL-60 (TB)	45.7	4.48	1.91
K-562	19.0	3.76	8.37
MOLT-4	10.9	5.98	0.336
RPMI-8226	7.58	3.08	11.7
SR	21.0	4.38	7.96
Non-small cell lung cancer			
EKVX	> 100	10.1	39.4
HOP-62	> 100	15.8	43.1
HOP-92	17.0	1.36	18.1
NCI-H226	> 100	20.6	46.0
NCI-H23	28.1	1.96	27.4
NCIH322M	> 100	3.42	38.7
NCI-H460	53.6	3.38	25.9
NCI-H 522	27.7	5.49	14.7
Colon cancer			
HCC-2998	31.1	3.60	19.6
HCT-116	14.8	2.74	12.8
HCT-15	15.9	3.71	19.7
HT29	28.6	3.18	16.7
KM12	83.8	3.21	22.9
SW-620	15.8	3.54	17.4
CNS cancer			
SF-268	20.6	2.83	22.7
SF-295	> 100	4.28	29.9
SF-539	> 100	5.59	44.9
U251	> 100	3.20	18.3
Melanoma			
LOX IMVI	29.3	3.92	21.9
M14	59.8	3.75	25.0
SK MEL-2	> 100	13.3	29.0
SK MEL-28	> 100	3.54	19.8
SK MEL-5	> 100	3.97	18.0
UACC-257	> 100	21.8	38.5
Ovarian cancer			
IGRVOI	> 100	3.80	21.0
OVCAR-3	48.1	17.1	30.5
OVCAR-4	22.5	9.85	19.5
OVCAR-5	89.0	3.96	24.1
OVCAR-8	24.7	6.02	18.8
SK-OV-3	> 100	19.3	50.9
Renal cancer			
786-O	24.5	4.23	28.0
A498	> 100	14.2	24.2
ACHN	48.4	4.65	33.5
CAKI-1	23.5	8.02	18.6
RXF-393	15.8	9.88	57.0
TK-10	86.7	11.6	32.6
UO-31	56.9	6.40	27.5
Prostate cancer			
PC-3	67.2	12.3	21.5
Du-145	71.6	5.97	17.4
Breast cancer			
MCF7	23.9	5.15	23.3
NCI/ADR-RES	17.9	4.24	33.2
MDA-MB-231/ATCC	> 100	3.72	29.7
HS-578T	51.1	11.8	15.3
MDA-MB-435	27.2	2.26	24.2
MDA-N	24.0	2.24	32.4
T-47D	44.3	13.6	20.4

The other two standard parameters, TGI and LC50 were above 100 μM (maximum tested concentration).

fluorophenyl)-3-(4-chloroaryloxy methyl)-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazine showed highest activity (MIC 6 μg ml^{-1}) against all the tested bacteria. The compound 7-(3,4-dimethoxy benzylidene)-6-(2,4-dichloro-5-fluorophenyl)-3-ethyl-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazine, which was emerged as most active compound in the earlier template exhibited MIC value 0.312 μg ml^{-1} against *E. coli*, *S. aureus* (Smith), *P. aeruginosa* (Gessard) and against *B. subtilis* 0.625 μg ml^{-1}. The results of this study is given in Table 4. With this result we can not arrive at any conclusion regarding SAR but for the same substituent 3,4-dimethoxy benzylidene at C7. This moiety may be contributing to their enhanced activity.

2.4. Antifungal activity

Newly prepared compounds were screened for their antifungal activity against *Candida albicans* (NCIM no. 3100) in DMSO by serial plate dilution method [12,13]. Activity of the compound was compared with amphotericin B as standard drug. The minimum inhibitory concentration (MIC) for the amphotericin B in DMSO is 1 μg ml^{-1} against the *C. albicans*. The antifungal study revealed that the tested compound **3** showed moderate to good antifungal activity with MIC value 6.0 μg ml^{-1} for most of the tested compounds. The results of this study is given in Table 5. Hence these series of compounds may emerge as good antifungal agents.

Table 4
Antibacterial activities of 7-arylidene-6-(2,4-dichloro-5-fluorophenyl)-3-(substituted)-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazines (**3**)

Compound numbers	MIC (μg ml^{-1})			
	E. coli	*S. aureus*	*P. aeruginosa*	*B. subtilis*
3a	6.0	12.5	12.5	6.0
3b	6.0	12.5	12.5	6.0
3c	6.0	12.5	12.5	6.0
3d	12.5	6.0	12.5	12.5
3e	12.5	6.0	12.5	12.5
3f	6.0	12.5	12.5	12.5
3g	12.5	12.5	12.5	12.5
3h	12.5	6.0	12.5	12.5
3i	6.0	6.0	6.0	6.0
3j	6.0	12.5	12.5	12.5
3k	6.0	6.0	6.0	6.0
3l	12.5	12.5	12.5	12.5
3m	6.0	6.0	6.0	6.0
3n	6.0	12.5	6.0	6.0
3o	12.5	6.0	12.5	12.5
3p	6.0	25.0	12.5	12.5
3q	6.0	12.5	6.0	6.0
3r	12.5	6.0	12.5	12.5
3s	12.5	12.5	12.5	6.0
3t	6.0	12.5	12.5	12.5
3u	6.0	12.5	12.5	12.5
3v	6.0	12.5	6.0	6.0
3w	12.5	25.0	12.5	12.5
3x	25.0	12.5	12.5	6.0
3y	12.5	12.5	12.5	12.5
3z	12.5	6.0	6.0	6.0
3a[1]	6.0	12.5	12.5	6.0
3b[1]	12.5	6.0	12.5	12.5
Nitrofurazone	6.0	12.5	–	12.5

Table 5
Antifungal activities of 7-arylidene-6-(2,4-dichloro-5-fluorophenyl)-3-(substituted)-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazines (**3**)

Compound numbers	MIC in μg ml^{-1} Zone of inhibition (in mm)
	C. albicans
3a	6.0 (15–20)
3b	6.0 (15–20)
3c	6.0 (15–20)
3d	6.0 (15–20)
3e	12.5 (< 10)
3f	6.0 (15–20)
3g	12.5 (< 8)
3h	12.5 (< 8)
3i	6.0 (15–20)
3j	12.5 (10–12)
3k	6.0 (15–20)
3l	12.5 (< 8)
3m	6.0 (15–20)
3n	6.0 (15–20)
3o	12.5 (< 8)
3p	6.0 (15–20)
3q	12.5 (< 8)
3r	6.0 (15–20)
3s	12.5 (< 8)
3t	6.0 (15–20)
3u	6.0 (15–20)
3v	12.5 (< 8)
3w	12.5 (< 8)
3x	12.5 (10–12)
3y	6.0 (15–20)
3z	12.5 (< 8)
3a[1]	6.0 (15–20)
3b[1]	12.5 (< 10)

3. Experimental

3.1. Chemistry

Melting points were taken in open capillary tubes and are uncorrected. IR spectra in KBr pellets were recorded on JASCO FT-IR 5300 Infrared Spectrophotometer. ^{1}H NMR spectra were recorded in DMSO-d_6 on a Varian (300 MHz) spectrometer using TMS as an internal standard and the mass spectra were recorded on aVG-s-70 micro mass, mass spectrometer operating at 70 eV. The purity of the compounds were checked by TLC using ethyl acetate/methanol [8:2] solvent system. Iodine was used as visualizing agent.

*3.1.1. General procedure for the synthesis of 3-aryl-1-(2,4-chloro5-fluorophenyl)-2-bromo-2-propen-1-one (**1**)*

A mixture of dibromopropanone (0.01 mol) and triethylamine (0.05 mol) in dry toluene (50 ml) was stirred for 24 hours. The precipitated triethylammoniumhydrobromide was filtered. The filtrate was concentrated under reduced pressure. The precipitated solid was filtered, dried and recrystallized from chloroform.

1a: 3-(3,4-Methylenedioxy phenyl)-1-(2,4-chloro-5-fluorophenyl)-2-bromo-2-propen-1-one, m.p.: 110–12 °C; Yield, 78%; yellow needles Analysis: Found (calculated): C: 45.81 (45.93), H: 1.95 (1.91).

1b: 3-(3,4-Dimethoxy phenyl)-1-(2,4-chloro-5-fluorophenyl)-2-bromo-2-propen-1-one, m.p.: 95–97 °C; Yield, 75%; yellow needles Analysis: Found (calculated): C: 47.35 (47.00), H: 2.68 (2.76).

1c: 3-(4-Chlorophenyl)-1-(2,4-chloro-5-fluorophenyl)-2-bromo-2-propen-1-one, m.p.: 115–18 °C; Yield, 75%; pale yellow needles Analysis: Found (calculated): C: 41.55 (42.00), H: 1.80 (1.86).

1d: 3-(4-Dichlorophenyl)-1-(2,4-chloro-5-fluorophenyl)-2-bromo-2-propen-1-one, m.p.: 76–78 °C; Yield, 74%; pale yellow needles Analysis: Found (calculated): C: 40.46 (40.63), H: 1.29 (1.35).

*3.1.2. General procedure for the synthesis of 4-amino-5-mercapto-3-aryloxymethyl/amilinomethyl-1,2,4-triazoles (**2**)*

A mixture of carboxylic acid (0.01 mol) and thiocarbohydrazide (0.01 mol) contained in round-bottomed flask was heated on a mantle until the contents melted. The mixture was maintained at this temperature for 15–20 min. The product obtained on cooling was treated with sodium bicarbonate solution to dissolve the unreacted carboxylic acid if any. It was then washed with water and collected by filtration. Then the product was recrystallized from a mixture of dioxane and ethanol to afford the title compounds.

2a: 3-(4-Chlorophenoxymethyl)-4-amino-5-mercapto-1,2,4-triazole, m.p. 183 °C (Ref. [14] m.p. 183–185 °C).

2b: 3-(2-Chlorophenoxymethyl)-4-amino-5-mercapto-1,2,4-triazole, m.p. 162 °C (Ref. [14] m.p. 162–165 °C).

2c: 3-(4-Chloro-3-methyl-phenoxymethyl)-4-amino-5-mercapto-1,2,4-triazole, m.p. 167 °C (Ref. [14] m.p. 167–169 °C).

2d: 3-(2,4-Dichloro-phenoxymethyl)-4-amino-5-mercapto-1,2,4-triazole, m.p. 186 °C (Ref. [14] m.p. 185–187 °C).

2e: 3-Anilinomethyl-4-amino-5-mercapto-1,2,4-triazole, m.p. 216 °C (Ref. [15] m.p. 215–217 °C).

2f: 3-(4-Chloroanilinomethyl)-4-amino-5-mercapto-1,2,4-triazole, m.p. 204 °C (Ref. [15] m.p. 204–206 °C).

2g: 3-(3-Chloro-4-fluoro-anilinomethyl)-4-amino-5-mercapto-1,2,4-triazole, m.p. 189–191 °C; Elemental analysis, Found (calculated): C: 41.39 (41.61), H: 3.39 (3.46) and N: 21.50 (21.57).

*3.1.3. Synthesis of 7-arylidene-6-(2,4-dichloro-5-fluorophenyl)-3-substitued 1,2,4-triazolo[3,4-b]-1,3,4-thiadiazines (**3a–3b[1]**)*

An equimolar mixture of 2-bromo-2-propen-1-one (**1**) and 3-substituted 4-amino-5-mercapto-1,2,4-triazole (**2**) and solution of potassium hydroxide (10%, 2.5 ml) in ethanol (25 ml) was kept under reflux on a water bath for about 5 h. The reaction mixture was cooled and the precipitated solid was filtered, washed with water, dried and recrystallized from ethanol and dioxane. The analytical data are given in Table 1.

3.2. Pharmacology

3.2.1. Anticancer activity

Eleven of the newly synthesized compounds **3** were screened for their antitumor activities at NIH, Bethesda, MD,

USA under the Drug Discovery Programme of NCI according to the procedure suggested by Boyd and Paull [10] in a primary three cell line-one dose anticancer assay against NCI-H 460 (lung), MCF 7(breast) and SF 268 (CNS). In the current protocol each cell line is inoculated on an incubated microtiter-plate. The test agents are added at a single concentration and the culture is incubated for 48 h. Endpoint determinations 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 2% or less (negative numbers indicate cell kill) are passed on for evaluation in the full panel of 60 cell lines over a 5-log dose range.

3.3. Antibacterial activity

The newly synthesized thiadiazines **3** were screened for their antibacterial activity against *E. coli*, *S. aureus* (Smith), *P aeruginosa* (Gessard) and *B. subtilis* bacterial strains by serial dilution method [11].

3.4. Antifungal activity

Sabourands agar media was prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in distilled water (100 ml) and adjusted the pH to 5.7. Normal saline was use to make a suspension of spores of fungal strain for lawning. A loopful of particular fungal strain was transferred to 3 ml saline to get a suspension of corresponding species. Agar media of 20 ml was poured in to each Petri dishes. Excess of suspension was decanted and the plates were dried by placing in an incubator at 37 °C for 1 h. Using an agar punch wells were made on these seeded agar plates and 5–100 μg ml^{-1} of the test compounds in DMSO were added in to each well labeled. A control was also prepared for the plates in the same way using solvent DMSO. The Petri dishes were prepared in triplicate and maintained at 37 °C for 3–4 days. Diameter of the inhibition zone and MIC were noted. The results of such studies are given in Table 5.

4. Conclusions

In a hope to improve antibacterial and anticancer activities of 3-alkyl/aryl-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazines **3**, we considered substitution at C3 by aryloxymethyl and anilinomethyl group for alkyl/aryl groups which was reported in our earlier work. But actually, we found that replacement of alkyl and aryl group by aryloxymethyl and anilinomethyl substituents was not advantageous in the case of antibacterial activity. In the case of antitumor activity only **3o**, **3m** and **3q** were emerged as active compounds whereas in the alkyl/aryl substituted triazolothiadiazine series, compounds with methyl at C3 and 3,4-methylenedioxy benzylidene at C7, methyl at C3 and 4-chloro benzylidene at C7, methyl at C3 and 3,4-dimethoxy benzylidene at C7 and phenyl group at C3 and 3,4-dimethoxy benzylidene at C7 were found to be active. Compound **3o** [2-chloroaryloxy methyl at C3 and 4-chloro benzylidene at C7 substitution] showed highest activity with GI_{50} value < 10 μM against all tested 60 cell lines. Whereas compound **3q** [4-chloro-3-methyl-aryloxy methyl at C3 and 4-chloro benzylidene at C7 substitution] showed moderate activity GI_{50} value < 50 μM against all tested 60 cell lines though it showed highest activity GI_{50} value = 0.336 μM against leukemia MOLT-4 cell line among active compounds. Compound **3m** [4-chloro-anilinomethyl at C3 and 3,4-dimethoxy benzylidene at C7 substitution] appears to be least active with GI_{50} value > 100 μM for some of the tested cell lines. As the compounds for the anticancer studies were selected randomly, we are unable to comment on SAR, because it is a preliminary study. However, it is hoped that triazolothiadiazines may emerge as potential compounds with varied biological activities in future.

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