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Facile synthesis and cytotoxic activity of 3,6-disubstituted 1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazoles

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ABSTRACT

A facile synthesis of 3,6-disubstituted-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazoles (5a-j) has been achieved by condensing 3-aryl substituted 4-amino-5-mercapto (4H)-1,2,4-triazole (4a-b) with various aromatic acids. The structures of the synthesized compounds were supported by IR, ^{1}H NMR, mass spectral data and elemental analysis The compounds were evaluated for *in vitro* cytotoxic activity against four human cancer cell lines: two ovarian (PA-1and OAW-42) and two breast (T47D and MCF-7) cell lines using the methyl thiazol tetrazolium assay method. It showed that some of the tested compounds 5a, 5b, 5g and 5f exhibited significant activity against PA-1 cell lines and the compounds 5a and 5e exhibited IC_{50} of 0.72 µM and 0.65 µM, respectively, against the cell lines of MCF-7, which are close to Doxorubin. After comparing the cytotoxic activity results of compounds 5a-j, it was concluded that the incorporation of triazolo-thiadiazole moiety in aryl propionic acid group gives rise to enhanced anticancer activity. Also, the substitution of chloro group in the aryl ring at the 3rd position was found to enhance their potency.

1. Introduction

Cancer remains a major public health issue at the beginning of the 21st century. Chemotherapy is one way to fight against cancer. Significant side effects such as nausea, vomiting, diarrhoea, hair loss and serious infections (mostly due to leucopoenia) often accompany chemotherapy. Therefore, the need for accelerated development of new, more effective as well as less toxic chemotherapeutic agents have appeared. Thiadiazoles have been of great interest as antitumor compounds for several scores of years [1-3]. Recent literature shows that 1,2,4-triazole and their fused heterocyclic derivatives have received considerable attention due to their synthesis and biological importance. For example, various 1,2,4-triazole derivatives have been reported to possess diversified biological properties such as antibacterial [4] antifungal [5], antiviral [6], antileishmanial [7] and antimigraine [8] activities. The available therapeutically important medicines Terconazole, Itraconazole, Fluconazole, Cefazoline, Ribavarin, Triazolam, Alprazolam, Etizolam and Furacylin [9] are some of the examples which contain anyone of these heterocyclic nucleuses. The fused 1,2,4–triazolo-[3,4-b]-1,3,4-thiadiazole derivatives shows various biological properties such as antifungal [10], antibacterial [11], antiviral [12], anthelmentic [13], antitumor [14] and anti-inflammatory activities [15]. Furthermore, literature survey revealed that modification of aryl propinic acid derivatives of nonsteroidal antiinflammatory drugs (NSAIDs) results in various biological activities such as anti-inflammatory, antibacterial and antiviral [16-17]. Therefore, it was considered worthwhile to replace the carboxylic acid group of 2-[(2',6'-dichlorophenyl)amino acetoxy acetic acid (Acelofenac) and 2-[(2',6'-dichlorophenyl)amino acetic acid (Diclofenac) by a composite system, which combine both the triazolo and thiadiazole nucleus in a ring to give a compact and planar structure. Thus, prompted by these finding, herein, we report the synthesis and *in vitro* cytotoxic activity of a series of 3,6-disubstituted 1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazole derivatives against breast and ovarian human cell lines using the methyl thiazol tetrazolium assay method (MTT) [18].

2. Experimental

2.1. Instrumentation

Melting point was determined on Veego digital melting point apparatus and was uncorrected. IR spectra were recorded using potassium bromide on a Perkin Elmer FT-IR Spectrophotometer. ^{1}H NMR spectra were recorded on Bruker Spectrophotometer (400 MHz) in $CDCl_3$ using TMS as an internal standard. Mass spectra were recorded on LC-MSD Trap-SL 2010A-Shimadzu. Micro analysis was performed on a Perkin Elmer-240 CHN elemental analyzer.

2.2. Synthesis

2.2.1. General Procedure for the synthesis of aryl ester (1a-b)

A solution of corresponding aromatic acids (0.01 mol) in 100 mL ethanol and 1 mL of sulphuric acid was refluxed for 6 h. The completion of the reaction was checked on precoated silica gel G plates using chloroform: methanol (9:1) as an eluent and observed under UV light (Figure 1). The solution was cooled, and the product obtained was collected by vacuum distillation and recrystallized from ethanol to yield compounds **1a-b**.

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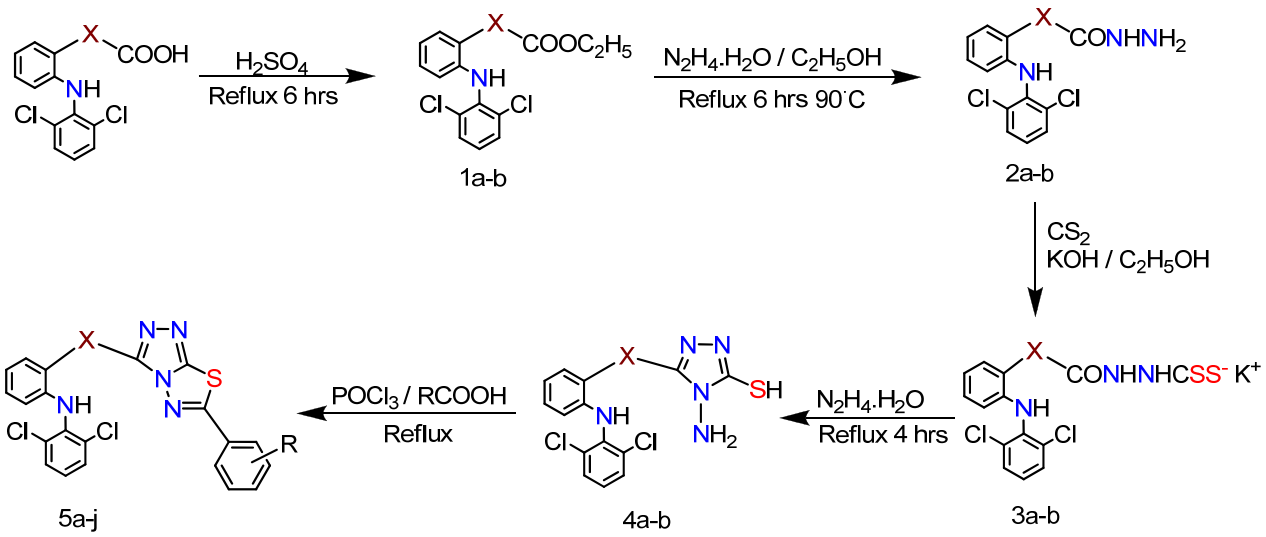

Figure 1. Synthesis of 3,6-disubstituted-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazole derivatives (**5a-j**).

2-[(2',6'-dichlorophenyl)amino]phenylacetoxyacetate (1a): Yield: 65 %. M.p.: 165-166 °C. Anal. Calcd. for $C_{18}H_{17}Cl_2NO_4$ (382.24): C, 59.53; H, 4.48; N, 3.66. Found: C, 59.45; H, 4.51; N, 4.01 %. FT-IR (cm^{-1}): *ν*(C=O) 1742; *ν*(NH) 3400; *ν*(C=C) 1614, 1531, 1487; *ν*(C-Cl) 790.

2-[(2',6'-dichlorophenyl)amino]phenylacetate (1b): Yield: 51 %. M.p.: 122-124 °C. Anal. Calcd. for $C_{16}H_{15}Cl_2NO_2$ (324.2): C, 59.28; H, 4.66; N, 4.32. Found: C, 59.35; H, 4.74; N, 4.43 %. FT-IR (cm^{-1}): *ν*(C=O) 1693; *ν*(NH) 3404; *ν*(C=C) 1623, 1491, 1462; *ν*(C-Cl) 796.

2.2.2. General Procedure for the synthesis of aryl hydrazide (2a-b)

A mixture of compound **1a/1b** (0.011 mol) and hydrazine hydrate (0.02 mol) in 50 mL methanol was heated under reflux for 5-6 h. The completion of the reaction was monitored on silica gel G precoated TLC plates using ethyl acetate and petroleum ether (1:1) as an eluent and observed under UV light. The reaction mixture was left overnight. Solid obtained was collected by flask evaporator and recrystallized from methanol.

2-[(2',6'-dichlorophenyl)amino]phenylacetoxyacetylhydrazide (2a): Yield: 75 %. M.p.: 190-196 °C. Anal. Calcd. for $C_{16}H_{15}Cl_2N_3O_3$ (368.21): C, 52.19; H, 4.11; N, 11.41. Found: C, 52.33; H, 4.24; N, 11.63 %. FT-IR (cm^{-1}): *ν*(NH_2) 3310; *ν*(NH) 3224; *ν*(C=O) 1711; *ν*(C-Cl) 781.

2-[(2',6'-dichlorophenyl)amino]phenylacetylhydrazide (2b): Yield: 67 %. M.p.: 173-174 °C. Anal. Calcd. for $C_{14}H_{13}Cl_2N_3O$ (310.18): C, 54.21; H, 4.22; N, 13.55. Found: C, 54.13; H, 4.36; N, 13.64 %. FT-IR (cm^{-1}): *ν*(NH_2)3342; *ν*(NH) 3251; *ν*(C=O) 1694; *ν*(C-Cl) 791.

2.2.3. General Procedure for the synthesis of 3-aryl substituted 4-amino-5-mercapto-(4H)-1,2,4-triazole (4a-b)

A solution of 50 mL of alcoholic potassium hydroxide (0.03 mol) was cooled in an ice bath and compound **2a/2b** (0.016 mol) was added with stirring. Then, carbon disulphide (0.025 mol) was added in small portions under constant stirring. The reaction mixture was agitated continuously for 12 h at room temperature. The precipitated potassium thiocarbamate **(3a-b)** was filtered, washed with ethanol, dried and directly used for the next step without further purification.

The above potassium thiocarbamate was mixed with water (8 mL) and hydrazine hydrate (0.02 mol) and refluxed for 4-5 h. During the progress, homogeneous reaction mixture which turned green with evolution of hydrogen sulphide gas was obtained. The reaction product was cooled to room temperature and diluted with water. On acidification with acetic acid, the required triazole **(4a-b)** precipitated out. The purity was checked by TLC using precoated silica gel G plate with toluene: ethyl acetate: formic acid (5:4:1) as solvent system and observed under UV light.

3-[2-[(2',6'-dichlorophenyl)amino]phenylacetoxymethyl]-4-amino-5-mercapto(4H)-1,2,4-triazole (4a): Yield: 66 %. M.p.: 158-159 °C. Anal. Calcd. for $C_{18}H_{17}Cl_2N_5O_2S$: C, 59.53; H, 4.48; N, 3.66. Found: C, 59.45; H, 4.51; N, 4.01 %. FT-IR (cm^{-1}): *ν*(NH_2) 3315; *ν*(SH) 2608; *ν*(NH-NH_2) 1605; *ν*(C-Cl) 781. ^{1}H NMR δ (ppm): 13.9 (s, 1H, S*H*), 7.2-8.0 (m, 7H, Ar*H*), 5.4 (s, 2H, NH_2), 3.45 (s, 2H, -OCH_2-), 5.1 (s, 2H, CH_2CO).

3-[2-[(2',6'-dichlorophenyl)amino]benzyl]-4-amino-5-mercapto(4H)-1,2,4-triazole (4b): Yield: 77 %; M.p.: 188-190 °C. Anal. Calcd. for $C_{15}H_{13}Cl_2N_5S$ (366.27): C, 49.19; H, 3.58; N, 19.12. Found: C, 49.41; H, 3.61; N, 19.09 %. FT-IR (cm^{-1}): *ν*(NH_2) 3400; *ν*(SH) 2618; *ν*(NH-NH_2) 1624; *ν*(C-Cl) 801. ^{1}H NMR δ (ppm): 13.6 (s, 1H, S*H*), 7.5-8.2 (m, 7H, Ar*H*), 5.9(s, 2H, NH_2), 2.3 (s, 2H, -CH_2-).

2.2.4. General method for the synthesis of 3,6-disubstituted-1,2,4-triazolo-[3, 4-b]-1,3,4-thiadiazoles (5a-j)

An equimolar mixture of compound **4a/4b** and appropriate aromatic acids in 10 mL phosphorus oxychloride was refluxed for 5 h. The reaction mixture was cooled to room temperature and poured onto crushed ice with stirring. Finally, to remove the excess of phosphorus oxychloride powdered potassium carbonate and the required amount of potassium hydroxide were added till the pH of the mixture was raised to 8. The solid was collected by vacuum distillation, dried and recrystallized from methanol.

3-[2-(2',6'dichlorophenyl)aminophenylacetoxymethyl]-6-(3-chlorophenyl)-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazole (5a): Yield: 70 %. M.p.: 184-185 °C. Anal. Calcd. for $C_{24}H_{16}Cl_3N_5O_2S$ (544.84): C, 52.91; H, 2.96; N, 12.85. Found: C, 52.71; H, 2.94; N, 12.72 %. FT-IR (cm^{-1}): *ν*(NH) 3284; *ν*(C=N) 1240; *ν*(C=O) 1732; *ν*(C=C) 1565; *ν*(C-Cl) 730. ^{1}H NMR δ (ppm): 6.75-8.15 (m, 11H, Ar*H*), 3.45 (s, 2H -OCH_2-), 5.2 (s, 2H, CH_2-CO), 4.15 (s, 1H, N*H*). MS (m/z): 543 (M-1).

3-[2-(2',6'dichlorophenyl)aminophenylacetoxymethyl]-6-(4-chlorophenyl)-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazole (5b): Yield: 65 %. M.p.: 190-191 °C. Anal. Calcd. for $C_{24}H_{16}Cl_3N_5O_2S$ (544.84): C, 52.91; H, 2.96; N, 12.85. Found: C, 52.89; H, 2.90; N, 12.79 %. FT-IR (cm^{-1}): *ν*(NH) 3298; *ν*(C=N) 1242; *ν*(C=O) 1709; *ν*(C=C) 1594; *ν*(C-Cl) 760. ^{1}H NMR δ (ppm): 7.2-8.1 (m, 11H, Ar*H*), 3.5 (s, 2H -OCH_2-), 5.45 (s, 2H, CH_2-CO), 4.2 (s, 1H, N*H*). MS (m/z): 543 (M-1).

3-[2-(2',6'dichlorophenyl)aminophenylacetoxymethyl]-6-(4-nitrophenyl)-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazole (5c): Yield: 66 %. M.p.: 176-178 °C. Anal. Calcd. for $C_{24}H_{16}Cl_2N_6O_4S$ (554.03): C, 51.9; H, 2.90; N, 15.14. Found: C, 52.06; H, 2.83; N, 15.24 %. FT-IR (cm^{-1}): *ν*(NH) 3078; *ν*(C=N) 1209; *ν*(C=O) 1759; *ν*(C=C) 1643; *ν*(C-NO_2) 1530, 1306; *ν*(C-Cl) 786. ^{1}H NMR δ (ppm): 6.9-8.5 (m, 11H, Ar*H*), 3.6 (s, 2H -OCH_2-), 5.3 (s, 2H, CH_2-CO), 4.3 (s, 1H, N*H*). MS (m/z): 554 (M^+).

3-[2-(2',6'dichlorophenyl)aminophenylacetoxymethyl]-6-(2-methoxyphenyl)-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazole (5d): Yield: 72 %. M.p.: 124-125 °C. Anal. Calcd. for $C_{25}H_{15}Cl_2N_5O_3S$ (540): C, 55.56; H, 3.54; N, 12.96. Found: C, 55.49; H, 3.60; N, 12.89 %. FT-IR (cm^{-1}): *ν*(NH) 3279; *ν*(C=N) 1208; *ν*(C=O) 1796; *ν*(C=C) 1617; *ν*(C-O-C) 1234, 1067; *ν*(C-Cl) 678. ^{1}H NMR δ (ppm): 6.5-8.2 (m, 11H, Ar*H*), 3.45(s, 2H, -OCH_2-), 3.7(s, 3H, OCH_3), 5.2(s, 2H, CH_2-CO), 4.4(s, 1H, N*H*). MS (m/z): 539 (M-1).

3-[2-(2',6'-dichlorophenyl)aminophenylacetoxymethyl]-6-(3,5-dichlorophenyl)-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazole (5e): Yield: 70 %. M.p.: 224-225 °C. Anal. Calcd. for $C_{24}H_{15}Cl_4N_5O_2S$ (579): C, 49.76; H, 2.61; N, 12.09. Found: C, 49.61; H, 2.70; N, 12.11 %. FT-IR (cm-^{1}): *ν*(NH) 3229; *ν*(C=N) 1239; *ν*(C=O) 1796; *ν*(C=C) 1576; *ν*(C-Cl) 765. ^{1}H NMR δ (ppm): 6.4-8.8 (m, 10H, Ar*H*), 3.55 (s, 2H, -OCH_2-), 5.5 (s, 2H, CH_2-CO), 4.3 (s, 1H, N*H*). MS(m/z): 578.97 (M-1).

3-[2-[(2',6'-dichlorophenyl)amino]benzyl]-6-(3-chlorophenyl)-1,2,4-triazolo-[3,4-b]-1,3, 4-thiadiazole (5f): Yield: 65%. M.p.: 165-166 °C. Anal. Calcd. for $C_{22}H_{14}Cl_3N_5S$ (486): C, 54.28; H, 2.90; N, 14.39. Found: C, 54.36; H, 3.07; N, 14.43 %. FT-IR (cm^{-1}): *ν*(NH) 3332; *ν*(C=N) 1259; *ν*(C=C) 1605; *ν*(C-Cl) 756. ^{1}H NMR δ (ppm): 7.6-8.4 (m, 11H, Ar*H*), 2.65 (s, 2H, CH_2-), 4.05 (s, 1H, N*H*). MS (m/z): 485 (M-1).

3-[2-[(2',6'-dichlorophenyl)amino]benzyl]-6-(4-chlorophenyl)-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazole (5g): Yield : 60 %. M.p.: 185-186 °C. Anal. Calcd. for $C_{22}H_{14}Cl_3N_5S$ (486): C, 54.28; H, 2.90; N, 14.39. Found: C, 54.39; H, 3.05; N, 14.37 %. FT-IR (cm^{-1}): *ν*(NH) 3302; *ν*(C=N) 1249; *ν*(C=C) 1585; *ν*(C-Cl) 775. ^{1}H NMR δ (ppm): 8.2-9.0 (m, 11H, Ar*H*), 2.55 (s, 2H, CH_2-), 4.3 (s, 1H, N*H*). MS(m/z): 485 (M-1).

3-[2-[(2',6'-dichlorophenyl)amino]benzyl]-6-(4-nitrophenyl)-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazole (5h): Yield: 75 %. M.p.: 218-219 °C. Anal. Calcd. for $C_{22}H_{14}Cl_2N_6O_2S$ (497): C, 53.16; H, 2.84; N, 16.90. Found: C, 53.31; H, 2.88; N, 16.72 %. FT-IR (cm^{-1}): *ν*(NH) 3276; *ν*(C=N) 1219; *ν*(C=C) 1652; *ν*(C-NO_2)1538, 1307; *ν*(C-Cl) 785. ^{1}H NMR δ (ppm): 7.7-9.1 (m, 11H, Ar*H*), 2.3 (s, 2H, CH_2), 4.1 (s, 1H, N*H*). MS (m/z): 496 (M-1).

3-[2-[(2',6'-dichlorophenyl)amino]benzyl]-6-(2-methoxyphenyl)-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazole (5i): Yield: 84 %. M.p.: 155-156 °C. Anal. Calcd. for $C_{23}H_{17}Cl_4N_5OS$ (482.3): C, 57.27; H, 3.55; N, 14.52. Found: C, 57.55; H, 3.62; N, 14.49 %. FT-IR (cm^{-1}): *ν*(NH) 3278; *ν*(C=N) 1209; *ν*(C=C) 1618; *ν*(C-O-C) 1229, 1059; *ν*(C-Cl) 785. ^{1}H NMR δ (ppm): 7.1-7.9 (m, 11H, Ar*H*), 3.7 (s, 3H, OCH_3) 2.6 (s, 2H, CH_2-), 4.6 (s, 1H, N*H*). MS (m/z): 481 (M-1).

3-[2-[(2',6'-dichlorophenyl)amino]benzyl]-6-(3,5-dichlorophenyl)-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazole (5j): Yield: 70 %. M.p.: 180-181 °C. Anal. Calcd. for $C_{22}H_{13}Cl_4N_5S$ (544.84): C, 52.91; H, 2.96; N, 12.85. Found: C, 52.71; H, 2.94; N, 12.72 %. FT-IR (cm^{-1}): *ν*(NH) 3226; *ν*(C=N) 1220; *ν*(C=C) 1586; *ν*(C-Cl) 768. ^{1}H NMR δ (ppm): 6.9-7.9 (m, 10H, Ar*H*), 2.4 (s, 2H, CH_2-), 4.1 (s, 1H, N*H*). MS (m/z): 543 (M-1).

2.3. In vitro cytotoxic assay

Cell line and culture: Tumor cell lines used in this study are two breast human cell lines T47-D (ductal carcinoma) and MCF-7 (adreno carcinoma) and two ovarian cancer cell lines PA-1 (terato carcinoma) and OAW-42 (ovarian cystaden ocarcinoma), respectively. All the cells were obtained from cell line bank of National Center of Cellular Sciences (NCCS), Pune, India. These cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) at pH 7.4, supplemented with 10 % heat-inactivated fetal bovine serum, 5 µM Ciprofloxacin and 40 µM Gentamycin in a humidified incubator at 37±0.5 °C and 5 % CO_2 atmosphere.

Evaluation of cytotoxicity: The cells from a particular cell line when in log phase of growth are trypsinized, counted in a haemocytometer and adjusted to 10^4 densities in a DMEM medium per plates and then, inoculated in 96-well plates (Tarsons Products Ltd, Kolkata). The cells are treated in 0.1-100 µM concentrations of test compounds for specified duration (1-4 days). After 10 µL of MTT dye was added in each well, plates were incubated at 37±0.5 °C for 4 hrs in a CO_2 incubator. The plates were then taken out of the incubator and dark blue colored formazan crystals are thoroughly dissolved in 100 µL of dimethyl sulphoxide (DMSO) at room temperature for 15 min. The absorbance values for each plate were then recorded on an ELISA reader at 550 nm. Doxorubicin 10 µM was applied as a standard drug. The 50 % of the cell viability (IC_{50}) was calculated and given in the Table 1.

3. Results and Discussion

The acid hydrazides (**2a-b**) were prepared by esterification of 2-[(2',6'-dichlorophenyl)amino]phenylacetoxyacetate and 2-[(2',6'-dichlorophenyl)amino]phenylacetate (**1a-b**) followed by treatment with hydrazine hydrate in absolute ethanol. The acid hydrazides were allowed to react with carbon disulphide in the presences of potassium hydroxide in ethanol to afford the corresponding intermediate potassium thiocarbamate (**3a-b**). This salt underwent ring closure with excess of hydrazine hydrate to give 3-aryl substituted-4-amino-5-mercapto-(4H)-1,2,4-triazole (**4a-b**). The resulted triazoles were further converted to 3,6-disubstituted-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazoles (**5a-j**) by condensing with various aromatic acids in the presence of phosphorus oxychloride as outlined in Figure 1. The structure of 3-aryl substituted-4-amino-5-mercapto-(4H)-1,2,4-triazole (**4a-b**) was confirmed by IR and ^{1}H NMR spectra. The ^{1}H NMR showed a downfield at 13.6-13.9 ppm attributed to the SH group, while the NH_2 group appeared as a singlet at 5.4-5.9 ppm. The absences of signals due to NH_2 and SH protons confirmed that the triazoles were converted into triazolo-thiadiazoles **5a-j** by reacting with COOH group of acids. Both analytical and spectral data (IR, ^{1}H NMR, MS and

Table1. In vitro cytotoxic activities of triazolo-thiadiazole compounds (**5a-j**) on growth of human tumor cell lines.

Compound	X	R	IC_{50} μM[a]		IC_{50} μM[a]	
			PA-1[b]	OAW-42[b]	T47 D[c]	MCF[c]
5a	CH_2COOCH_2-	-3-chloro	0.63	72	20	0.70
5b	-CH_2COOCH_2	-4-chloro	0.72	76	24	0.95
5c	-CH_2COOCH_2	-4-nitro	1.25	>100	>100	24
5d	-CH_2COOCH_2	-2-methoxy	2.50	84	>100	54
5e	-CH_2COOCH_2	-3,5-dichloro	0.85	86	29	0.65
5f	-CH_2-	-3-chloro	0.74	78	25	1.50
5g	-CH_2	-4-chloro	0.96	87	27	0.83
5h	-CH_2	-4-nitro	1.02	>100	75	20
5i	-CH_2	-2-methoxy	3.52	76	61	68
5j	-CH_2	-3,5-dichloro	0.92	87	74	25
Doxorubin	-	-	0.74	0.62	0.77	0.72

[a] 50 % cell viability concentration in micromol (μM).
[b] Human ovarian cancer cell lines.
[c] Human breast cancer cell lines.

elemental analysis) of all the synthesized compounds were in full agreement with the proposed structure.

The new triazolo-thiadiazole derivatives (**5a-j**) were evaluated for *in vitro* cytotoxic activity by methyl thiazol tetrazolium (MTT) assay method against four human cancer cell lines; two ovarian (PA-1 and OAW-42) and two breast (T47D and MCF-7) cell lines. The IC_{50} values were calculated by dose depended curve and given in Table 1. It showed that some of the tested compounds (**5a, 5b** and **5g**) exhibited higher inhibitory activity and **5f** showed equipotent activity against PA-1 cell line in comparison with the standard drug Doxorubicin (10 μM). Similarly, the compounds **5a** and **5e** exhibited higher cytotoxic activity against the ovarian cancer cell lines of MCF. The results of the biological activity revealed that the replacement of the halo phenyl group in the 6th position of the triazolo-thiadiazole gives rise to more active compounds. All the synthesized compounds showed moderate activity against the cell lines of OAW-42 and T47D. After comparing the cytotoxic activity results of compounds **5a-j**, it was concluded that the incorporation of triazolo-thiadiazole moiety in aryl propionic acid group gives rise to enhanced anticancer activity. Also, the substitution of chloro group in the aryl ring at 3rd position was found to enhance their potency especially in compound **5a** against the PA-1 and MCF human cancer cell lines. Substitution of triazolo-thiadiazole in Aceclofenac moiety exhibited higher activity which may be due to the presence of a bulky group in that position. Further studies to acquire structural activity relationship are in progress in our laboratory.

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