Synthesis of New *S*-alkylated-3-mercapto-1,2,4-triazole Derivatives Bearing Cyclic Amine Moiety as Potent Anticancer Agents

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Received August 01, 2011: Revised November 04, 2011: Accepted November 28, 2011

Abstract: A series of 3-[3-[4-(Substituted)-1-cyclicamine]propyl]thio-5-substituted[1,2,4]triazoles (**8a-j**) were synthesized with good yields starting from corresponding carboxylic acids. The cytotoxicity studies of these derivatives were studied against five different human cancer cell lines. Three compounds had shown good anticancer activity. The triazole derivatives, **8i** and **8j** were most potent particularly against U937 and HL-60 cells. The cytotoxic potency of the compounds varied between the cell lines suggesting that a structural property of these compounds as possible determinants of their biological activity.

Keywords: 1,2,4-Triazoles, Acid hydrazides, Piperazines, Anticancer.

INTRODUCTION

Cancer drug discovery has traditionally focused on targeting DNA synthesis, angiogenesis and cell division [1]. Although existing drugs show efficacy, their lack of selectivity for tumor cells over normal cells can lead to severe side effects [2]. Design and synthesis of novel small molecules which can specifically block some targets in tumor cells are in perspective direction in modern medicinal chemistry. Hence, there is an urgent need for the discovery and development of new anticancer agents. From different groups of heterocycles, many synthetic small molecules with cytotoxic activity have been reported and several of them have undergone the clinical trials [3].

The chemistry of 1,2,4-triazoles and their fused heterocyclic derivatives has received considerable attention in the last few decades, owing to their synthetic and effective biological importance [4]. The 1*H*-1,2,4-triazole compounds possess an important pharmacological activities such as, anti-inflammatory, CNS stimulants sedatives, antianxiety, antimicrobial [5,6], and antimycotic agents such as fluconazole, intraconazole and voriconazole [7]. Also, there are known drugs containing the 1,2,4-triazole group e.g. Triazolam [8], Alprazolam [9], Etizolam, and Furacylin.

Moreover, sulphur containing heterocycles represent an important group of compounds and these heterocycles i.e., the mercapto- and thione-substituted 1,2,4-triazole ring systems exhibit biological activities, such as anticancer [10,11], antitubercular [12], diuretic [13], antibacterial [14], antifungal [15], antimycobacterial [16], and hypoglycemic [17].

Several piperazine derivatives were synthesized in the last decade, as useful chemotherapeutic agents for various diseases, such as crivixan (indinavir sulphate) and delaviridine (rescriptor), powerful inhibitors for the protease and reverse transcriptase inhibitor of HIV enzymes, respectively [18]. Some piperizinyl [1,2,4]triazole derivatives were reported as 5-HT_{1A} serotonin receptor ligands [19].

In view of these facts, the aim of the present study was to synthesize new compounds containing 1,2,4-triazole and piperazine moieties in a single molecular framework as possible anticancer agents. In the present work we have described synthesis of 3-[3-[4-(substituted)-1-cyclicamine] propyl]thio-5-substituted[1,2,4]triazoles (**8**). The cytotoxic activity of these derivatives was studied against five different types of human cancer cell lines i.e. U937, THP-1, Colo205, MCF7 and HL-60.

RESULTS AND DISCUSSION

Chemistry

5-Substituted [1,2,4]triazole-3-thiones (**5**) were synthesized by following reported sequence of the reactions from corresponding aryl carboxylic acids (**1**) (Scheme **1**). The aryl carboxylic acids (**1**) were converted to corresponding methyl esters (**2**) with catalytic amount of sulfuric acid in methanol. This was confirmed by disappearance of broad carboxylic acid peak in ^{1}H NMR spectra and disappearance of broad 'OH' stretching of carboxylic acid in IR spectra of the product. It was also supported by appearance of OCH_3 peak in ^{1}H NMR spectra of methyl esters (**2**). The methyl esters (**2**) were converted to corresponding acid hydrazides (**3**) using hydrazine hydrate in methanol [20]. This was confirmed by disappearance of -$OC\underline{H}_3$ peak and appearance of N<u>H</u> and $N\underline{H}_2$ peaks in ^{1}H NMR spectra of the product. It was also supported by the appearance of NH stretching and C=O stretching of acid hydrazide in IR spectra of the product. The acid hydrazides (**3**) were reacted with potassium thiocyanate and concentrated hydrochloric acid to produce corresponding thiosemicarbazides (**4**) [21]. Appearance of additional NH

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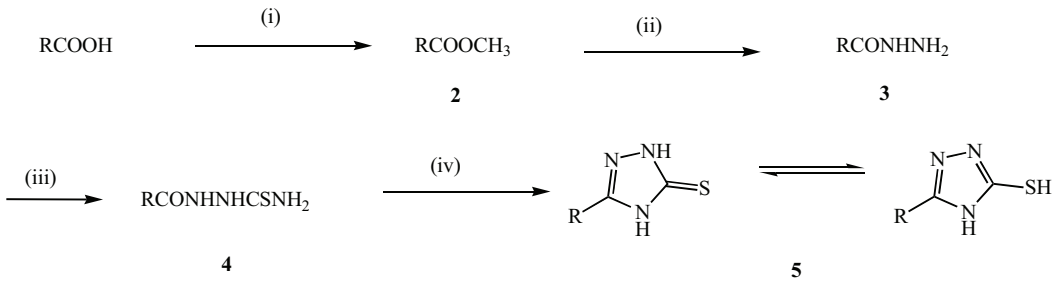
1875-628X/12 $58.00+.00



R = 4-Cl-C_6H_4, 3-Cl-C_6H_4, 2-Cl-C_6H_4, 4-CH_3-C_6H_4, 3-CH_3-C_6H_4, 4-F-C_6H_4, 4-C_4H_9-C_6H_4, 4-OCH_3-C_6H_4, $C_6H_5CH_2$, 3,4-di OCH_3-C_6H_3.

Reagents and conditions: (i) Cat. H_2SO_4, CH_3OH, reflux, 4 h; (ii) $NH_2NH_2.H_2O$, CH_3OH, reflux, 2-3 h; (iii) KSCN/ Conc. HCl, CH_3OH, reflux, 1 h; (iv) 10% Aqueous NaOH, CH_3OH, reflux, 5-6 h.

Scheme 1. Synthesis of 5-substituted[1,2,4]triazole-3-thiones (**5**).

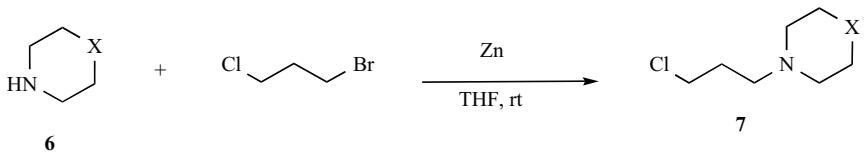
stretching in IR spectra and molecular weight confirmed the formation of thiosemicarbazides (**4**). Thiosemicarbazides (**4**) were reacted with aqueous sodium hydroxide to produce 5-substituted[1,2,4]triazole-3-thiones (**5**) [22]. They can also exist in tautomeric thiol form. Here we found that they mainly exist in thione form which is also supported by some earlier reports [4]. Some reports state that they mainly exist in thione-thiol tautomeric forms in solution but the thione structures dominate in the solid state [23]. It has been reported that the crystal structure of these types of compounds corresponded to the thione form [24]. The product was confirmed by appearance of characteristic broad peak for NH protons with chemical shift 13-14 ppm. Appearance of characteristic triazole NH stretching in IR spectra and molecular weight had further confirmed the formation of 5-substituted[1,2,4]triazole-3-thiones (**5**).

Now, 1-(3-Chloropropyl)-4-substituted cyclic amines (**7**) were prepared by the reaction of cyclic amines (**6**) with 1-bromo-3-chloropropane in presence of activated zinc in THF at ambient temperature (Scheme **2**) [25].

When the 5-substituted[1,2,4]triazole-3-thiones (**5**) and 1-(3-chloropropyl)-4-substituted cyclic amines (**7**) were reacted in the presence of triethyl amine in ethanol with a catalytic amount of tetra butyl ammonium iodide (TBAI) the compounds 3-[3-[4-(substituted)-1-cyclic amine]propyl]thio-5-substituted[1,2,4]triazoles (**8**) were formed (Scheme **3**). In the derivatives **8**, a triplet for the methylene group of the propyl chain that connects the cyclic amine moiety and the triazole part of the molecule is observed with a chemical shift in the range of δ 3.25-3.15, which is typical for S–C<u>H</u>$_2$ connectivity [19, 26]. Further the products **8a-j** were confirmed by mass and IR spectroscopy and the details are given in Table **1**.

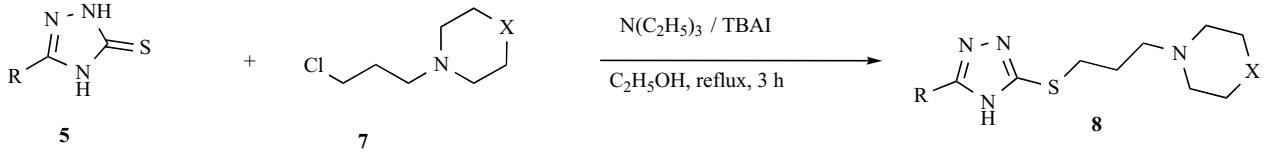
Cytotoxicity Studies

Cytotoxicty was measured using the MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide] assay, according to the method of Mossman [27]. The Triazole derivatives **8a-j** were tested for *in vitro* cytotoxic activity against U937, THP-1, Colo205, MCF7 and HL-60 human cancer cell lines. IC_{50} values of the test compound for 24 h on each cell line was calculated and presented in Table **2**. It is evident from the results that the triazole derivatives were found to be more potent on U937 and HL-60 cells than



X= O, N-ethyl, *N*-phenyl, *N*-benzyl, *N*-2-pyrimidyl, *N*-2-pyridyl, *N*-3-chlorophenyl

Scheme 2. Synthesis of 1-(3-chloropropyl)-4-substituted cyclic amines (**7**).



R = 4-Cl-C_6H_4, 3-Cl-C_6H_4, 2-Cl-C_6H_4, 4-CH_3-C_6H_4, 3-CH_3-C_6H_4, 4-F-C_6H_4, 4-C_4H_9-C_6H_4, $C_6H_5CH_2$

X= O, *N*-ethyl, *N*-phenyl, *N*-benzyl, *N*-2-pyrimidyl, *N*-2-pyridyl, *N*-3-chlorophenyl

Scheme 3. Synthesis of 3-[3-[4-(substituted)-1-cyclic amine]propyl]thio-5-substituted[1,2,4]triazoles (**8**).

Table 1. 3-[3-[4-(Substituted)-1-cyclic amine]propyl]thio-5-substituted[1,2,4]triazoles (8a-j)

Entry	Compound	R	X	Yield (%)
1	8a	4-C_4H_9-C_6H_4	O	72
2	8b	4-C_4H_9-C_6H_4	N-phenyl	66
3	8c	4-CH_3-C_6H_4	N-3-chlorophenyl	70
4	8d	4-CH_3-C_6H_4	N-ethyl	63
5	8e	4-Cl-C_6H_4	N-3-chlorophenyl	75
6	8f	4-Cl-C_6H_4	N-2-pyrimidyl	65
7	8g	4-F-C_6H_4	N-benzyl	70
8	8h	3-CH_3-C_6H_4	N-phenyl	66
9	8i	3-CH_3-C_6H_4	N-2-pyridyl	70
10	8j	3-Cl-C_6H_4	N-2-pyrimidyl	68

other three cell lines of THP-1, Colo205 and MCF-7. Some of the triazole derivatives had shown significant decrease in cell viability in some of the test cell lines on concentration dependent manner. Etoposide was used as a positive control in these assays and the IC_{50} values obtained on U937, THP-1, Colo205, MCF-7 and HL-60 as 10.43, 3.66, 12.3, 29.49 and 1.84 μM respectively.

The triazole derivatives **8j, 8i** and **8f,** were potent antiproliferative agents against U937 cells, with IC_{50} values of 49.13 μM, 52.33 μM and 102.24 μM, respectively. Interestingly, similar trend was observed even in HL-60 cells, with IC_{50} values of 18.51 μM, 29.36 μM and 105.06 μM, respectively. The triazole derivative **8b** was active against U937 (IC_{50} 49.31) and THP-1 (IC_{50} 97.63). Very few of the traizole derivatives (**8a**, **8c** and **8g**) were active against Colo205 and MCF-7.

Overall it was observed from the results that cytotoxicity depends on the particular substituents on the phenyl ring and piperizine ring. These findings suggest that the newly synthesized triazole have shown significant anti-proliferative activity on U937 and HL-60 cells than other three cell lines of THP-1, Colo205 and MCF-7. The cytotoxic potency of the compounds varied between the cell lines suggesting that a structural property of these compounds as possible determinants of their biological activity.

CONCLUSIONS

Synthesis of 3-[3-[4-(substituted)-1-cyclicamine]propyl] thio-5-substituted[1,2,4]triazoles (**8a-j**) was achieved with good yields starting from corresponding carboxylic acids. The anticancer activity of these derivatives was studied. Among the compounds (Entry: 1-10) tested for anticancer activity, majority of the compounds have exhibited moderate to good anticancer activity. The triazole derivatives, **8j** and **8i** were the most potent particularly against U937 and HL-60 cells. The cytotoxic potency of the compounds varied between the cell lines suggesting that a structural property of these compounds as possible determinants of their biological activity.

Table 2. Cytotoxic Activity (IC_{50}, μM) of Compounds 8a-j Against Human Cancer Cell Lines

Compound	IC_{50}[a] (μM)				
	U937	THP-1	COLO 205	MCF7	HL-60
8a	156.11±1.47	----	155.36±9.4	286.8±35.0	---
8b	49.31±3.97	97.63±11.8	----	----	---
8c	256.84±39.01	----	139.29±0.15	----	---
8d	----	----	----	----	---
8e	----	----	----	----	---
8f	102.24±5.59	---	---	---	105.06±5.49
8g	124.0.3±6.61	255.42±8.85	181.41±4.28	184.42±2.86	---
8h	----	247.55±15.26	----	----	---
8i	52.33±3.12	---	---	---	29.36±2.23
8j	49.13±2.86	---	---	---	18.51±1.16
Etoposide[b]	10.43±2.0	3.66±0.25	12.3±2.14	29.49±2.22	1.84±0.20

Exponentially growing cells were treated with different concentrations of triazole derivatives for 24 h and cell growth inhibition was analyzed through MTT assay.
[a]IC_{50} is defined as the concentration, which results in a 50% decrease in cell number as compared with that of the control cultures in the absence of an inhibitor and were calculated using the respective regression analysis. The values represent the mean ± SE of three individual observations.
[b]Etoposide was employed as positive control.
--- indicates IC_{50} value >300 μM.

EXPERIMENTAL

Chemistry

The ^{1}H NMR (200 MHz) spectra were recorded on Varian Gemini spectrometer using tetra methyl silane as an internal standard. *J* values are given in Hz. IR spectra were recorded on Perkin-Elmer Infrared-683. Mass spectra were recorded on Agilent Technologies LC/MSD SL single quadrupole (Agilent ChemStation software).

General Procedure for the Preparation of Methyl Esters (2)

A catalytic amount of concentrated H_2SO_4 was added to a solution of an appropriate aryl carboxylic acid **1** (0.15 mol) in 150 mL of methanol and the mixture was refluxed for 4 h. It was allowed to cool. The saturated solution of $NaHCO_3$ was added to the reaction mixture and was extracted with methylene dichloride (CH_2Cl_2) (2 X 100 mL). The combined organic layer was dried (Na_2SO_4) and concentrated to obtain pure methyl ester (**2**) and the yields are obtained quantitatively.

General Procedure for the Preparation of Acid Hydrazides (3) [20]

To a solution of an appropriate methyl ester **2** (0.1 mol) in 150 mL of methanol was added 95% hydrazine hydrate (0.2 mol), and the mixture was refluxed for 2-3 h upto reaction completed (TLC). It was allowed to cool, and the obtained solid was washed with methanol and dried (Na_2SO_4). The crude compounds were recrystalized from ethanol to obtain the product in more than 90% yields.

General Procedure for the Preparation of Thiosemicarbazides (4) [21]

To a solution of an appropriate acid hydrazide **3** (0.02 mol) in 50 mL of methanol was added a solution of KSCN (0.03 mol) and 3 mL of HCl with constant stirring. The mixture was immediately evaporated to dryness on a steam bath and heated for an additional 1 h with another 50 mL of methanol. The resulting solid was treated with distilled water and with a small amount of ethanol. The crude thiosemicarbazides (**4**) were used as such for next step.

General Procedure for the Preparation of 5-substituted [1,2,4]triazole-3-thiones (5) [22]

To a solution of an appropriate thiosemicarbazide **4** (0.01 mol) in 15 mL of methanol was added a solution of 10.0% NaOH (20 mL), and the reaction mixture was refluxed immediately for 5-6 h upto reaction was completed (TLC). The mixture was cooled and acidified with dilute HCl at pH 5-6. The resulting solid was filtered, washed with distilled water, and dried (Na_2SO_4). The crude compounds were recrystalized from ethanol to obtain pure product in more than 90% yield.

General Procedure for the Preparation of 1-(3-chloropropyl)-4-substituted Cyclic Amines (7) [25]

The activated zinc powder (0.002 mol) was added to a solution of cyclic amine **6** (0.002 mol) and 1-bromo-3-chloropropane (0.002 mol) in THF (20 mL) and stirred at room temperature for 2 h. After completion of the reaction, the mixture was filtered and the solid was washed with solvent ether (3 X 20 mL). The combined filtrate was treated with 10% $NaHCO_3$ (20 mL), water (2 X 20 mL), dried (Na_2SO_4) and evaporated to give the crude product. The crude product was purified by flash column chromatography. The pure products were obtained in the range 70-85% yields

General Procedure for the Preparation of 3-[3-[4-(substituted)-1-cyclic amine]propyl]thio-5-substituted[1,2,4]triazoles (8a-j)

5-Substituted [1,2, 4]triazole-3-thione **5** (0.0005 mol) was refluxed with triethyl amine (0.001 mol) in ethanol (8 mL) for 15 minutes. 1-(3-Chloropropyl)-4-substituted cyclic amine **7** (0.0006 mol) and tetra butyl ammonium iodide (TBAI) (10 mg) in ethanol (3 mL) were added to above mixture carefully and was refluxed for 4 h. After the reaction was completed, water (10 mL) was added and extracted with ethyl acetate (3 X 10 mL). The combined organic layer was dried (Na_2SO_4) and concentrated. The crude product was subjected to flash column chromatography to obtain pure product and the yields of the products were mentioned in Table **1**.

Spectoscopic Data for the Synthesized Compounds

5-[4-(Tert-butyl)phenyl]-4H-1,2,4-Triazol-3-yl (3-morpholinopropyl) sulfide (8a)

Brown gummy liquid. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.90 (d, J= 8.6, 2H, 2XAr-H); 7.42 (d, J= 8.6, 2H, 2XAr-H); 3.69-3.57 (m, 4H, 2XO-CH_2); 3.17 (t, J= 7.0 Hz, 2H, S-CH_2); 2.51-2.32 (m, 6H, 3XN-CH_2); 1.91 (quintet, J= 7.0 Hz, 2H, $CH_2C\mathbf{H_2}CH_2$); 1.34 (s, 9H, $C(CH_3)_3$). IR (KBr, cm^{-1}): 3152 (N-H), 2959, 2862, 2812 (-C-H), 1615 (C=C), 1456, 1329 (C-N), 1267, 1117 (C-O), 847, 756 (=C-H ben). ESI-MS (m/z): 361 $(M+1)^+$.

5-[4-(Tert-butyl) phenyl]-4H-1,2,4-Triazol-3-yl [3-(4-phenylpiperazino)propyl] sulfide (8b)

White solid. mp 138-140 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.90 (d, J= 8.3 Hz, 2H, 2XAr-H); 7.35 (d, J= 8.3 Hz, 2H, 2XAr-H); 7.16 (t, J = 7.9 Hz, 2H, 2XAr-H); 6.83-6.75 (m, 3H, 3XAr-H); 3.26-3.16 (m, 4H, 2XN-CH_2); 3.13 (t, J= 6.8 Hz, 2H, S-CH_2); 2.76-2.68 (m, 4H, 2XN-CH_2); 2.64 (t, J= 6.6 Hz, 2H, N-CH_2); 1.99-1.94 (m, 2H, $CH_2C\mathbf{H_2}CH_2$); 1.29 (s, 9H, $C(CH_3)_3$). IR (KBr, cm^{-1}): 3448 (N-H), 2956 (-C-H), 1639 (C=C), 1495, 1420, 1225 (C-N), 768 (=C-H ben). ESI-MS (m/z): 436 $(M+1)^+$.

3-[4-(3-Chlorophenyl)piperazino]propyl [5-(4-methylphenyl)-4H-1,2,4-triazol-3-yl] sulfide (8c)

Yellow solid. mp 85-86 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.88 (d, J= 7.8 Hz, 2H, 2XAr-H); 7.21 (d, J= 7.8 Hz, 2H, 2XAr-H); 7.12 (t, J = 8.6 Hz, 1H, Ar-H); 6.85-6.67 (m, 3H, 3XAr-H); 3.28-3.04 (m, 6H, S-CH_2 & 2XN-CH_2); 2.67-2.45 (m, 6H, 3XN-CH_2); 2.40 (s, 3H, Ar-CH_3); 1.97 (quintet, J=7.0 Hz, 2H, $CH_2C\mathbf{H_2}CH_2$). IR (KBr, cm^{-1}): 2947, 2826 (-C-H), 1594 (C=C), 1486, 1328, 1274 (C-N), 834, 760 (=C-H ben). ESI-MS (m/z): 428 (M^+).

3-(4-Ethylpiperazino)propyl [5-(4-methylphenyl)-4H-1,2,4-Triazol-3-yl] sulfide (8d)

Brown gummy liquid. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.90 (d, J= 7.6 Hz, 2H, 2XAr-H); 7.16 (d, J= 8.3 Hz, 2H, 2XAr-H); 3.15 (t, J= 6.8 Hz, 2H, S-CH_2); 2.72-2.40 (m, 12H,

6XN-CH_2); 2.37 (s, 3H, Ar-CH_3); 2.02-1.93 (m, 2H, $CH_2C\mathbf{H_2}CH_2$); 1.26 (t, J= 6.8 Hz, 3H, $C\mathbf{H_3}CH_2$). IR (KBr, cm^{-1}): 3406 (N-H), 2930, 2823 (-C-H), 1615 (C=C), 1450, 1327, 1267 (C-N), 830, 752 (=C-H ben). ESI-MS (m/z): 346 $(M+1)^+$.

3-[4-(3-Chlorophenyl)piperazino]propyl [5-(4-chlorophenyl)-4H-1,2,4-triazol-3-yl] sulfide (8e)

White solid. mp 72-74 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.96 (d, J= 8.8 Hz, 2H, 2XAr-H); 7.33 (d, J= 8.1 Hz, 2H, 2XAr-H); 7.18-7.04 (m, 1H, Ar-H); 6.94-6.62 (m, 3H, 3XAr-H); 3.44-3.04 (m, 6H, S-CH_2 & 2XN-CH_2); 2.77-2.45 (m, 6H, 3XN-CH_2); 2.00 (quintet, J= 7.3 Hz, 2H, $CH_2C\mathbf{H_2}CH_2$). IR (KBr, cm^{-1}): 3077 (C-N), 2942, 2825 (-C-H), 1594 (C=C), 1486, 1327, 1239 (C-N), 839, 755 (=C-H ben). ESI-MS (m/z): 448 (M^+).

5-(4-Chlorophenyl)-4H-1,2,4-triazol-3-yl 3-[4-(2-pyrimidinyl)piperazino]propyl sulfide (8f)

White solid. mp 128-129 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 8.24 (d, J= 5.1 Hz, 2H, 2XAr-H); 7.99 (d, J= 8.9 Hz, 2H, 2XAr-H); 7.40 (d, J= 8.9 Hz, 2H, 2XAr-H); 6.47 (t, J= 5.1 Hz, 1H, Ar-H); 3.85-3.74 (m, 4H, 2XN-CH_2); 3.23 (t, J= 7.4 Hz, 2H, S-CH_2); 2.57-2.30 (m, 6H, 3XN-CH_2); 1.97 (quintet, J= 7.4 Hz, 2H, $CH_2C\mathbf{H_2}CH_2$). IR (KBr, cm^{-1}): 3171 (N-H), 2930, 2852 (-C-H), 1586, 1493 (C=C), 1448, 1260 (C-N), 752 (=C-H ben). ESI-MS (m/z): 417 $(M+1)^+$.

3-(4-Benzylpiperazino)propyl [5-(4-fluorophenyl)-4H-1,2,4-triazol-3-yl] sulfide (8g)

Brown gummy liquid. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 8.08-7.94 (m, 2H, 2XAr-H); 7.33-7.16 (m, 5H, 5XAr-H); 7.11-6.97 (m, 2H, 2XAr-H); 3.54 (s, 2H, Ph-CH_2); 3.12 (t, J= 6.0 Hz, 2H, S-CH_2); 2.72-2.43 (m, 10H, 5XN-CH_2); 1.97 (quintet, J= 6.0 Hz, 2H, $CH_2C\mathbf{H_2}CH_2$). IR (KBr, cm^{-1}): 3405 (N-H), 2932, 2830 (-C-H), 1606 (C=C), 1455, 1326, 1226 (C-N), 847, 752 (=C-H ben). ESI-MS (m/z): 412 $(M+1)^+$.

5-(3-Methylphenyl)-4H-1,2,4-Triazol-3-yl [3-(4-phenylpiperazino)propyl] sulfide (8h)

White solid. mp 75-77 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.88-7.74 (m, 2H, 2XAr-H); 7.37-7.10 (m, 4H, 4XAr-H); 6.89-6.70 (m, 3H, 3XAr-H); 3.27-3.05 (m, 6H, S-CH_2 & 2XN-CH_2); 2.62-2.45 (m, 6H, 3XN-CH_2); 2.40 (s, 3H, Ar-CH_3); 1.95 (quintet, J= 7.0 Hz, 2H, $CH_2C\mathbf{H_2}CH_2$). IR (KBr, cm^{-1}): 3421 (N-H), 2921, 2852 (-C-H), 1599 (C=C), 1456, 1328, 1234 (C-N), 741, 685 (=C-H ben). ESI-MS (m/z): 394 $(M+1)^+$.

5-(3-Methylphenyl)-4H-1,2,4-Triazol-3-yl 3-[4-(2-pyridyl) piperazino]propyl sulfide (8i)

White solid. mp 109-101 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 8.14 (dd, J= 3.8, 1.5 Hz, 1H, Ar-H); 7.94-7.74 (m, 2H, 2XAr-H); 7.42 (t, J= 7.6 Hz, 1H, Ar-H); 7.22 (t, J= 7.6 Hz, 1H, Ar-H); 7.12 (d, J= 6.8 Hz, 1H, Ar-H); 6.61-6.54 (m, 2H, 2XAr-H); 3.665-3.57 (m, 4H, 2XN-CH_2); 3.17 (t, J= 6.8 Hz, 2H, S-CH_2); 2.62-2.52 (m, 6H, 3XN-CH_2); 2.34 (s, 3H, Ar-CH_3); 1.97 (quintet, J= 6.0 Hz, 2H, $CH_2C\mathbf{H_2}CH_2$). IR (KBr, cm^{-1}): 3067 (N-H), 2926, 2828 (-C-H), 1595 (C=C), 1483, 1247 (C-N), 772 (=C-H ben). ESI-MS (m/z): 395 $(M+1)^+$.

5-(3-Chlorophenyl)-4H-1,2,4-Triazol-3-yl 3-[4-(2-pyrimidinyl)piperazino]propyl sulfide (8j)

White solid. mp 115-117 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 8.24 (d, J= 4.7 Hz, 2H, 2XAr-H); 8.06-8.00 (m, 1H, Ar-H); 7.97-7.89 (m, 1H, Ar-H); 7.42-7.30 (m, 2H, 2XAr-H); 6.45 (t, J= 4.7 Hz, 1H, Ar-H); 3.88-3.75 (m, 4H, 2XN-CH_2); 3.25 (t, J= 7.0 Hz, 2H, S-CH_2); 2.59-2.34 (m, 6H, 3XN-CH_2); 1.98 (quintet, J= 7.0 Hz, 2H, $CH_2C\mathbf{H_2}CH_2$). IR (KBr, cm^{-1}): 3448 (N-H), 2924, 2846 (-C-H), 1587 (C=C), 1485, 1255 (C-N), 795 (=C-H ben). ESI-MS (m/z): 417 $(M+1)^+$.

Cytotoxicity Studies

Cell Lines and Cell Culture

The cell lines U937 (human leukemic monocytic lymphoma), THP-1 human acute monocytic leukemia), Colo205 (human colorectal cancer), MCF7 (human breast adenocarcinoma) and HL-60 (human myeloid leukemia) were obtained from the National Centre for Cellular Sciences (NCCS), Pune, India. Cells were cultured either in RPMI - 1640 (U937, THP-1, Colo205 and HL-60) or MEM (MCF7) media, supplemented with 10% heat- inactivated fetal bovine serum (FBS), 1 mM $NaHCO_3$, 2 mM -glutamine, 100 units/ml penicillin and 100 µg/ml streptomycin. All cell lines were maintained in culture at 37° C in an atmosphere of 5% CO_2.

Test Concentrations

Initially, stock solutions of each test substances were prepared in 100% Dimethyl Sulfoxide (DMSO, Sigma Chemical Co., St. Louis, MO) with a final concentration of 8 mg/ml. Exactly 150 µl of stock was diluted to 1 ml in culture medium to obtain experimental stock concentration of 1200 µg/ml. This solution was further serially diluted with media to generate a dilution series of 20 µg/ml to 600 µg/ml. Exactly 100 µl of each diluent was added to 100 µl of cell suspension (total assay volume of 200 µl) and incubated for 24 h at 37 °C in 5% CO_2.

Cytotoxicity

Cytotoxicty was measured using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay, according to the method of Mossman [27]. Briefly, the cells (2×10^4) were seeded in each well containing 0.1 ml of medium in 96 well plates. After overnight incubation at 37 °C in 5% CO_2, the cells were treated with 100 µl of different test concentrations of test compounds (2 to 60 µg) at identical conditions with five replicates each. The final test concentrations were equivalent to 10 to 300 µg/ml or 10 to 300 ppm. The cell viability was assessed after 24 h, by adding 10 µl of MTT (5 mg/ml) per well. The plates were incubated at 37 °C for additional three hours. The medium was discarded and the formazan blue, which formed in the cells, was dissolved with 100 µl of DMSO. The rate of color formation was measured at 570 nm in a spectrophotometer (Spectra MAX Plus; Molecular Devices; supported by SOFTmax PRO-5.4). The percent inhibition of cell viability was determined with reference to the control values (without test compound). The data were subjected to linear regression analysis and the regression lines were plotted for the best straight-line fit. The IC_{50} (inhibition of cell viability)

concentrations were calculated using the respective regression equation.

CONFLICT OF INTEREST

Declared none.

ACKNOWLEDGEMENTS

KRR, RVR, RP and LRV are thankful to Council of Scientific & Industrial Research (CSIR), New Delhi, India for the award of fellowships.

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