

Original Article

DESIGN, SYNTHESIS AND *IN VITRO* ANTI-CANCER ACTIVITY OF NOVEL 1,2,4-TRIAZOLE DERIVATIVES

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Received: 19 Jun 2014 Revised and Accepted: 01 Aug 2014

ABSTRACT

Objective: DNA topoisomerase is one of the important targets for anticancer agents. Many triazole derivatives have been shown to possess cytotoxic activity. In this paper, we present the design and *in silico* docking of a virtual library of molecules with DNA topoisomerase II along with their synthesis and *In vitro* cytotoxicity profile.

Methods: Sybyl X 2.1 programmess were used to perform the docking experiments on DNA topoisomerase II using etoposide as ligand. *In vitro* anticancer activity was carried out by trypan blue exclusion assay against EAC cells. DNA fragmentation studies were performed by Gel electrophoresis to identify the cause of cell death induced by these compounds.

Results: Among the compounds studied for docking, **12c** generated the highest docking score (13.66) and showed hydrogen bonding interactions with glycine 778 at a distance of 1.879 Å. the compounds **12c** & **12g** showed the highest level of cytotoxicity with IC₅₀ value of 0.55 µM and 0.62 µM respectively. Compounds **12c** and **12g** were subjected to DNA fragmentation studies to identify the cause of cell death induced by these compounds. Gel electrophoresis of these compounds showed a typical feature of apoptosis ladders in agarose gel. Compound **12c** was able to induce apoptosis at a concentration of about 3 µM.

Conclusion: A series of bis-triazoles were synthesized targeted to DNA topoisomerase II and evaluated their *In vitro* cytotoxicity. The compound **12c** was found to be most active and also exhibited apoptosis inducing potential.

Keywords: DNA Topoisomerase, Bis-triazole, Cytotoxicity, DNA fragmentation, Apoptosis.

INTRODUCTION

Nitrogen-containing five-membered heterocycles play a vital role in drug discovery to identify novel chemical entities of immense therapeutic potential. Triazoles are the most privileged structures that are widely explored for their range of pharmacological properties [1-4]. The application of anastrozole and letrozole as aromatase inhibitors for the treatment of estrogen-dependent cancer as well as the anticancer properties of ribavirin led to the investigation of many 1,2,4 triazole derivatives in laboratorial conditions for their antitumor activity [5-8]. Among the 1,2,4 triazole derivatives, the mercapto and thione substituted 1,2,4 triazole ring systems were reported to possess a variety of anti-tumor properties [9-14]. In literature, 1,2,4-triazoles are well documented for their broad spectrum of biological properties, including antifungal [15], antiviral [16], antimicrobial [17], A2A receptor antagonists [18], and COX-2 inhibitors [19]. Additionally 1,2,4 triazole derivatives have been reported to inhibit several enzymes which play an important role in the expression of tumors such as Protein Kinase CK2 [20], methionine aminopeptidase type II [21-22], Janus kinase 2 [23], Tankyrases [24-25]. Recently 1,2,4 triazoles have been identified as a new class of tubulin polymerization inhibitors [26]. In view of the previous rationale and in continuation of an on-going program

aiming at finding new structural leads with potential chemotherapeutic activities, a new series of triazole derivatives have been synthesized and screened for their anticancer activity.

MATERIALS AND METHODS

Design and Virtual Screening of Molecules

The designing and docking studies were carried out by using SYBYL X 2.1 (CERTARA, St Louis, Montana, USA). The protein structure of human Topoisomerase- II (TP II) was obtained from the protein data bank (PDB Code: 3QX3). This structure is determined at 2.16Å resolution. This structure of TP II is complexed with the TP II inhibitor etoposide. We performed the minimization of human TP II with 3QX3 as template using the 'prepare protein' module in SYBYL. The ligand substructure was extracted and all unnecessary water molecules were removed. Explicit hydrogen's were added to the protein and protein model was charged with Gasteiger-Marsili and ligand using Gasteiger-Huckel charges. Energy minimization and relaxation of the loop region were performed using 1000 iterations with AMBER7 FF99 as force field. All the molecules were docked into the active site of Human TP-II. The results of docking study are given in **Table 1**. The hydrogen bonding interactions of compounds in the active site of Topoisomerase II is depicted in **Fig. 1**.

Table 1: Docking Score expressed as total score for the docked compounds.

S. No.	Name	Total_Score	Crash	Polar
Std	Etoposide	13.72	-1.3661	3.7162
1	12c	11.72	-1.6505	0.8132
2	12g	11.67	-2.5205	0.5037
3	12a	11.65	-1.8578	3.2051
4	12b	11.44	-2.5574	0.5988
5	12d	11.43	-3.0563	1.1692
6	12h	11.41	-1.7074	3.5283
7	12i	11.38	-3.7593	0.5528
8	12e	11.36	-2.4724	1.0666
9	12f	11.32	-2.7468	0.9162



Fig. 1: (a)Etoposide showing hydrogen bonding interactions in the active site of DNA topoisomerase II, (b) Compound 12c in the active site of Topoisomerase II.

Chemistry

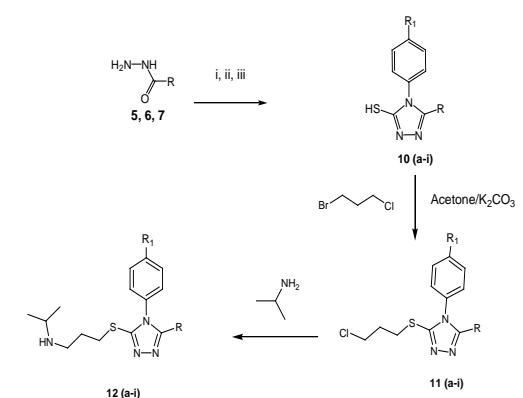
The reagents used for synthesis were of laboratory grade and solvents were of analytical grade. The melting point of the compound was determined by open capillary method, expressed in °C. The reaction was monitored periodically through TLC with the solvent system hexane: ethyl acetate in the ratio of 2:3 and hexane: methanol in the ratio of 3:2.

IR spectra were recorded on Shimadzu FT-IR 8400-S spectrophotometer by KBr pellet technique and are expressed in cm^{-1} . $^1\text{H-NMR}$ spectra was recorded on Bruker 400 MHz FT-NMR spectrophotometer using DMSO and CDCl_3 as solvent and TMS as internal standard (δ ppm). The chemical shifts are expressed in δ ppm. The splitting patterns were designated as follows; s: singlet; d: doublet; q: quartet; m: multiplet. $^{13}\text{C-NMR}$ spectra were recorded on Bruker 400 MHz FT-NMR spectrophotometer using DMSO as solvent. Mass spectra were recorded using Shimadzu Electron Impact Mass Spectrophotometer. All chemicals used for synthesis were purchased from (Sigma-Aldrich).

Synthesis

The strategy adopted to synthesize the molecules is depicted in **Scheme 1**. The aryl carbohydrazide (5-7) were converted to 3,4-diaryl, 5 mercapto 1,2,4-triazoles (**10 a-i**) by base catalysed cyclization of respective thiosemicarbazides. The triazoles are then treated with 1-bromo-3-chloro propane followed by isopropylamine to yield the 3-(4,5-substituted)-4H-1,2,4-triazol-3-ylthio)-N-isopropylpropan-1-amine derivatives (**12a-i**).

SCHEME - 1



i = Reflux with aryl isothiocyanates in ethanol; ii = Heat with 2N NaOH; iii = Neutralize with 2N HCl

Chemistry

General procedure for the synthesis of 3-(4,5-substituted)-4H-1,2,4-triazol-3-ylthio)-N-isopropylpropan-1-amine 12 (a-i).

To a solution of appropriate 4,5-(substituted)-4H-1,2,4-triazole-3-thiol(10 a-i) (0.001 mol) in 10 ml acetone, 1-bromo-3-chloropropane (0.001 mol, 0.15 ml) was added and the mixture was stirred under reflux for 4–8 h in the presence of potassium carbonate (0.01 mol, 1.37g). The reaction was monitored by TLC using a mixture of ethyl acetate & hexane in 3:2 ratio as the mobile phase. The excess of acetone was removed under reduced pressure.

The precipitate formed was washed with petroleum ether to obtain 3-(3-chloropropylthio)-4,5-disubstituted-4H-1,2,4-triazole (**11 a-i**) derivatives.

To a solution of appropriate 3-(3-chloropropylthio)-4,5-disubstituted-4H-1,2,4-triazole derivatives (**11 a-i**) (0.001 mol) in 10 ml methanol, isopropylamine (0.004 mol, 0.23 ml) was added and refluxed for 8-10 h at 70-80°C. The reaction was monitored by TLC using a mixture of hexane: methanol in 3:2 ratio as mobile phase. The mixture was then acidified with 2N hydrochloric acid and washed with ether. To the resulting aqueous phase 2N sodium hydroxide was added at 0°C. The solid residue was washed with water, dried and recrystallized from cyclohexane to obtain 3-(4,5-substituted)-4H-1,2,4-triazol-3-ylthio)-N-isopropylpropan-1-amine (**12 a-i**) derivatives.

3-(4-phenyl-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-ylthio)-N-isopropyl propan-1-amine (12a)

Yield 44%, mp: 209-211°C; IR (KBr) $\nu(\text{cm}^{-1})$: 1511 (Ar C=C), 3011 (Ar C-H), 2941 (Ali C-H), 3320 (N-H), 1161 (C-N); $^1\text{H NMR}$ (CDCl_3): (ppm) 1.27 (6H, d, $-\text{CH}(\text{CH}_3)_2$), 1.89 (2H, p, $-\text{CH}_2-\text{CH}_2-\text{CH}_2-$), 2.26 (1H, q, $-\text{CH}_2-\text{NH}-$), 2.71 (2H, q, $-\text{CH}_2-\text{NH}$), 3.45 (2H, t, $-\text{S}-\text{CH}_2-$), 3.92 (1H, m, $-\text{NH}-\text{CH}(\text{CH}_3)_2$), 7.21-8.56 (9H, m, Ar-H); $^{13}\text{C NMR}$ (CDCl_3): (ppm) 21.4, 32.3, 35.4, 43.1, 47.7, 119.8, 128.1, 128.7, 129.6, 134.2, 145.5, 149.1, 155.2; EI-MS m/z (%) 353.13 (M^+ , 42); Anal. Calcd (%) for $\text{C}_{19}\text{H}_{23}\text{N}_5$: C, 64.56; H, 6.56; N, 19.81; Found: C, 64.45; H, 6.56; N, 19.76.

3-(4-(4-chlorophenyl)-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-ylthio)-N-isopropylpropan-1-amine (12b)

Yield 38%, mp: 219-222°C; IR (KBr) $\nu(\text{cm}^{-1})$: 1538 (Ar C=C), 3018 (Ar C-H), 2960 (Ali C-H), 3476 (N-H), 1049 (C-N), 679 (C-Cl); $^1\text{H NMR}$ (CDCl_3): (ppm) 1.17 (6H, d, $-\text{CH}(\text{CH}_3)_2$), 1.83 (2H, p, $-\text{CH}_2-\text{CH}_2-\text{CH}_2-$), 2.12 (1H, q, $-\text{CH}_2-\text{NH}-$), 2.54 (2H, q, $-\text{CH}_2-\text{NH}$), 3.57 (2H, t, $-\text{S}-\text{CH}_2-$), 3.82 (1H, m, $-\text{NH}-\text{CH}(\text{CH}_3)_2$), 7.12-8.69 (8H, m, Ar-H); $^{13}\text{C NMR}$ (CDCl_3): (ppm) 25.5, 29.9, 32.7, 45.8, 48.3, 121.6, 126.2, 128.2, 130.1, 133.7, 148.4, 150.7, 154.3; EI-MS m/z (%) 387.19 (M^+ , 48); Anal. Calcd (%) for $\text{C}_{19}\text{H}_{22}\text{ClN}_5$: C, 58.83; H, 5.72; N, 18.05; Found: C, 58.87; H, 5.79; N, 17.91.

3-(4-(4-fluorophenyl)-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-ylthio)-N-isopropylpropan-1-amine (12c)

Yield 45%, mp: 230-233°C; IR (KBr) $\nu(\text{cm}^{-1})$: 1595 (Ar C=C), 3011 (Ar C-H), 2970 (Ali C-H), 3390 (N-H), 1025 (C-N), 1242 (C-F); $^1\text{H NMR}$ (CDCl_3): (ppm) 1.23 (6H, d, $-\text{CH}(\text{CH}_3)_2$), 1.96 (2H, p, $-\text{CH}_2-\text{CH}_2-\text{CH}_2-$), 2.19 (1H, q, $-\text{CH}_2-\text{NH}-$), 2.61 (2H, q, $-\text{CH}_2-\text{NH}$), 3.51 (2H, t, $-\text{S}-\text{CH}_2-$), 3.73 (1H, m, $-\text{NH}-\text{CH}(\text{CH}_3)_2$), 7.34-8.54 (8H, m, Ar-H); $^{13}\text{C NMR}$ (CDCl_3): (ppm) 23.5, 30.1, 34.7, 45.4, 47.7, 115.8, 122.1, 125.7, 131.3, 134.2, 147.4, 149.7, 153.6, 162.7; EI-MS m/z (%) 371.19 (M^+ , 32); Anal. Calcd (%) for $\text{C}_{19}\text{H}_{22}\text{FN}_5$: C, 61.43; H, 5.97; N, 18.85. Found: C, 61.27; H, 5.89; N, 18.92.

3-(5-(phenoxyethyl)-4-phenyl-4H-1,2,4-triazol-3-ylthio)-N-isopropylpropan-1-amine (12d)

Yield 33%, mp: 222-225°C; IR (KBr) $\nu(\text{cm}^{-1})$: 1518 (Ar C=C), 3017 (Ar C-H), 2983 (Ali C-H), 3412(N-H), 1186 (C-N); $^1\text{H NMR}$ (CDCl_3): (ppm) 1.10 (6H, d, $-\text{CH}(\text{CH}_3)_2$), 1.81 (2H, p, $-\text{CH}_2-\text{CH}_2-\text{CH}_2-$), 2.11 (1H, q, $-\text{CH}_2-\text{NH}-$), 2.55 (2H, q, $-\text{CH}_2-\text{NH}$), 3.17 (2H, t, $-\text{S}-\text{CH}_2-$), 3.45 (1H, m, $-\text{NH}-\text{CH}(\text{CH}_3)_2$), 5.11 (2H, s, $-\text{O}-\text{CH}_2-$), 6.75-7.35 (10H, m, Ar-H); $^{13}\text{C NMR}$ (CDCl_3): (ppm) 24.5, 30.9, 34.6, 44.9, 49.7, 66.4, 114.1, 121.7, 126.7, 128.1, 129.7, 148.1, 151.7, 161.6; EI-MS m/z (%) 382.25 (M^+ , 65); Anal. Calcd (%) for $\text{C}_{21}\text{H}_{26}\text{N}_4\text{O}$: C, 65.94; H, 6.85; N, 14.65; Found: C, 65.82; H, 6.93; N, 14.49.

3-(4-(4-chlorophenyl)-5-(phenoxyethyl)-4H-1,2,4-triazol-3-ylthio)-N-isopropylpropan-1-amine (12e)

Yield 44%, mp: 247-251°C; IR (KBr) $\nu(\text{cm}^{-1})$: 1645 (Ar C=C), 3066(Ar C-H), 2963 (Ali C-H), 3413 (N-H), 1062 (C-N), 660 (C-Cl); $^1\text{H NMR}$ (CDCl_3): (ppm) 1.17 (6H, d, $-\text{CH}(\text{CH}_3)_2$), 1.76 (2H, p, $-\text{CH}_2-\text{CH}_2-\text{CH}_2-$), 2.32 (1H, q, $-\text{CH}_2-\text{NH}-$), 2.57 (2H, q, $-\text{CH}_2-\text{NH}$), 3.42 (2H, t, $-\text{S}-\text{CH}_2-$), 3.96 (1H, m, $-\text{NH}-\text{CH}(\text{CH}_3)_2$), 5.75 (2H, s, $-\text{O}-\text{CH}_2-$), 6.95-7.65 (9H, m, Ar-H); $^{13}\text{C NMR}$ (CDCl_3): (ppm) 25.7, 32.2, 36.5, 43.6, 48.4, 67.4, 114.9, 122.1, 125.7, 127.3, 128.2, 129.7, 135.7, 149.1, 153.6, 162.7;

El-MS m/z (%) 416.20 (M⁺, 40); Anal. Calcd (%) for C₂₁H₂₅ClN₄OS: C, 60.49; H, 6.04; N, 13.44; Found: C, 60.43; H, 6.09; N, 13.49.

3-(4-(4-fluorophenyl)-5-(phenoxy)methyl)-4H-1,2,4-triazol-3-ylthio)-N-isopropylpropan-1-amine (12f)

Yield 49%, mp: 239-242 °C IR (KBr) ν (cm⁻¹): 1654 (Ar C=C), 3007 (Ar C-H), 2973 (Ali C-H), 3392 (N-H), 1186 (C-N), 1064 (C-F); ¹H NMR (CDCl₃): (ppm) 1.29 (6H, d, -CH(CH₃)₂), 1.86 (2H, p, -CH₂-CH₂-CH₂-), 2.35 (1H, q, -CH₂-NH-), 2.69 (2H, q, -CH₂-NH), 3.45 (2H, t, -S-CH₂-), 3.93 (1H, m, -NH-CH-(CH₃)₂), 5.57 (2H, s, -O-CH₂-), 6.77-7.72 (9H, m, Ar-H); [13]C NMR (CDCl₃): (ppm) 25.5, 30.1, 36.7, 45.4, 47.9, 68.7, 114.9, 115.8, 122.1, 124.7, 129.4, 131.3, 147.4, 149.7, 160.6, 163.3; El-MS m/z (%) 400.26 (M⁺, 44); Anal. Calcd (%) for C₂₁H₂₅FN₄OS: C, 62.98; H, 6.29; N, 13.99; Found: C, 62.87; H, 6.35; N, 14.12.

3-(5-((4-chlorophenoxy)methyl)-4-phenyl-4H-1,2,4-triazol-3-ylthio)-N-isopropylpropan-1-amine (12g)

Yield 52%, mp: 211-214 °C IR (KBr) ν (cm⁻¹): 1518 (Ar C=C), 3017 (Ar C-H), 2965 (Ali C-H), 3430 (N-H), 1102 (C-N), 757 (C-Cl); ¹H NMR (CDCl₃): (ppm) 1.17 (6H, d, -CH(CH₃)₂), 1.93 (2H, p, -CH₂-CH₂-CH₂-), 2.16 (1H, q, -CH₂-NH-), 2.78 (2H, q, -CH₂-NH), 3.36 (2H, t, -S-CH₂-), 3.51 (1H, m, -NH-CH-(CH₃)₂), 5.21 (2H, s, -O-CH₂-), 6.85-7.56 (9H, m, Ar-H); [13]C NMR (CDCl₃): (ppm) 23.1, 30.7, 33.4, 46.2, 48.6, 115.8, 126.1, 127.7, 128.6, 129.9, 147.4, 150.7, 159.5; El-MS m/z (%) 416.19 (M⁺, 55); Anal. Calcd (%) for C₂₁H₂₅ClN₄OS: C, 60.49; H, 6.04; N, 13.44; Found: C, 60.43; H, 6.09; N, 13.39.

3-(5-((4-chlorophenoxy)methyl)-4-(4-chlorophenyl)-4H-1,2,4-triazol-3-ylthio)-N-isopropylpropan-1-amine (12h)

Yield 36%, mp: 219-222 °C IR (KBr) ν (cm⁻¹): 1584 (Ar C=C), 3006 (Ar C-H), 2954 (Ali C-H), 3412 (N-H), 1082 (C-N), 712 (C-Cl); ¹H NMR (CDCl₃): (ppm) 1.33 (6H, d, -CH(CH₃)₂), 1.89 (2H, p, -CH₂-CH₂-CH₂-), 2.30 (1H, q, -CH₂-NH-), 2.85 (2H, q, -CH₂-NH), 3.43 (2H, t, -S-CH₂-), 3.93 (1H, m, -NH-CH-(CH₃)₂), 5.75 (2H, s, -O-CH₂-), 6.71-7.53 (8H, m, Ar-H); [13]C NMR (CDCl₃): (ppm) 26.4, 31.1, 37.7, 42.4, 49.7, 64.2, 115.1, 124.1, 125.7, 129.2, 131.3, 135.7, 147.4, 149.7, 162.7; El-MS m/z (%) 450.19 (M⁺, 62); Anal. Calcd (%) for C₂₁H₂₄Cl₂N₄OS: C, 55.87; H, 5.36; N, 12.41; Found: C, 55.83; H, 5.32; N, 12.49.

3-(5-((4-chlorophenoxy)methyl)-4-(4-fluorophenyl)-4H-1,2,4-triazol-3-ylthio)-N-isopropylpropan-1-amine (12i)

Yield 42%, mp: 229-231 °C IR (KBr) ν (cm⁻¹): 1511 (Ar C=C), 3012 (Ar C-H), 2941 (Ali C-H), 3470 (N-H), 1161 (C-N), 605 (C-Cl), 1260 (C-F); ¹H NMR (CDCl₃): (ppm) 1.23 (6H, d, -CH(CH₃)₂), 1.96 (2H, p, -CH₂-CH₂-CH₂-), 2.19 (1H, q, -CH₂-NH-), 2.61 (2H, q, -CH₂-NH), 3.51 (2H, t, -S-CH₂-), 3.73 (1H, m, -NH-CH-(CH₃)₂), 5.65 (2H, s, -O-CH₂-), 6.78-7.34 (8H, m, Ar-H); [13]C NMR (CDCl₃): (ppm) 23.5, 30.1, 34.7, 45.4, 47.7, 65.5, 115.8, 122.1, 126.7, 131.3, 134.2, 148.6, 150.7, 157.4, 162.5; El-MS m/z (%) 434.27 (M⁺, 59); Anal. Calcd (%) for C₂₁H₂₄ClFN₄OS: C, 57.99; H, 5.56; N, 12.88; Found: C, 57.91; H, 5.59; N, 12.89.

In vitro cytotoxicity activity by Trypan Blue exclusion assay

The ascetic fluid withdrawn from the peritoneum of EAC bearing animal, was washed with PBS and cell viability was checked by trypan blue dye using haemocytometer.

From the stock cell suspension, 1*10⁶EAC cells in a volume of 0.1 ml were taken in sterile test tubes. The cells were treated with different concentration of compounds 0 μ M, 0.1 μ M, 0.2 μ M, 0.4 μ M, 0.6 μ M, 0.8 μ M, 1.0 μ M in (0.2% DMSO) in a volume of 0.1 ml. The volume was made up to 1 ml with PBS and incubated at 37°C for 3 hours [27-28].

After 3 hours of exposure, 0.1 ml of trypan blue was added, mixed well and the total number of dead and living cells in all the four corner squares of the chambers was counted using haemocytometer and the percentage cytotoxicity was calculated as follows:

$$\% \text{ Cytotoxicity} = (T_{\text{total}} - C_{\text{dead}}) / T_{\text{total}} \times 100$$

The drug concentration that causes 50% cell growth inhibition after 3h of continuous exposure to the test compounds (IC₅₀) was determined by plotting the graph of concentration of the drug against the percent cytotoxicity and performing the regression analysis. The IC₅₀ values of the test compounds are shown in Table 2.

Table 2: In vitro cytotoxicity of 1,2,4-triazole derivatives against EAC cells

S. No.	Compound	IC ₅₀ (μ M)
1	12a	0.80+0.015
2	12b	0.73+0.32
3	12c	0.55+ 0.09
4	12d	1.24+0.45
5	12e	2.21+0.52
6	12f	5.91+0.41
7	12g	0.62+ 0.23
8	12h	1.89+0.55
9	12i	2.38+0.17

DNA fragmentation studies

DNA fragmentation study was performed by agarose gel electrophoresis[29]. Gel electrophoresis was carried out on i-Mupid mini- gel electrophoresis unit from Eurogentech. The DNA ladder (180bp) used for the study was obtained from Sigma Aldrich.

Agarose gel was prepared by boiling agarose in TAE buffer. The proper comb was inserted into the gel rig. 5 μ l of ethidium bromide was added to the gel and allowed to cool to 55°C. The comb was then placed in the gel tray. The gel was then poured into the gel tray and allowed to set for 30 minutes. Once the gel had cooled the comb was then removed carefully to form eight wells. The gel tray was placed in the gel electrophoresis unit with the wells closest to the cathode. The gel rig was then filled with TAE buffer until it formed a layer of buffer above the gel. The DNA was then treated with different concentration of compounds 1 μ M, 2 μ M, 4 μ M, 6 μ M, 8 μ M, 10 μ M and incubated in a shaker water bath at 37°C for 3 hours. 20 μ l of sample was transferred to an eppendorf containing 2 μ l of gel loading dye. The first well was loaded with 10 μ l of 1 Kb ladder. The remaining wells were loaded with 10 μ l of DNA containing varying concentration of drug. The cover was placed on the gel rig. The power pack was set at 60V for 120 minutes. The run was stopped when the loading dye had migrated to about 3/4th of the gel tray. The gel tray was carefully removed and placed in a UV transilluminator to see the migration of DNA. The photographs were then taken using Gel Dock instrumentation (Biorad).

The cytotoxic profile indicated that compounds 12c and 12g possessed highest cytotoxicity against EAC cells. Compounds 12c and 12g were selected for DNA fragmentation study against the EAC cells using gel electrophoresis and the fragmentation was compared with the standard 180bp DNA ladder. The gel dock image of the activity is represented in Figure 2.

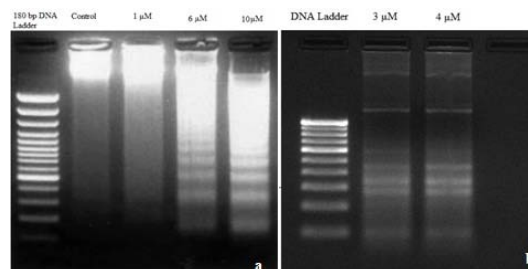


Fig. 2: DNA fragmentation profile of compound 12g (a) and 12c (b).

RESULTS AND DISCUSSION

Docking

Energy minimized 3D structure of Etoposide showed that the A & C rings oriented perpendicular to ring B. Etoposide exhibited a docking score of 13.72. The oxygen atom in the methoxy group of the phenyl ring in etoposide formed H-bonding with -NH group of glycine 778 at a distance of about 1.813 Å. Oxygen atom in the

dioxane ring of etoposide formed H-bonding with aspartic acid 479 at a distance of about 1.940 Å (Figure 1a).

Docking study of our synthesized compounds against DNA TP II showed that compound 12c penetrated and positioned at the same binding site of the receptor as of Etoposide (Figure 1b). Compound 12c exhibited the highest docking score of 11.72. Additionally, the superimposition of compound 12c with native ligand did not show any conformational change. In compound 12c, nitrogen atom of isopropyl amine group exhibited hydrogen bonding with the -NH group of glycine 778 at a distance of 1.879 Å. This is similar to the hydrogen bonding exhibited by etoposide varying only by a distance of about 0.064 Å. The higher cytotoxic action of compound 12c could be due to its resemblance with docking interactions of etoposide.

In vitro cytotoxicity by Trypan Blue exclusion assay

In vitro cytotoxicity by Trypan blue exclusion assay showed that all compounds possessed good cytotoxic activity against EAC cells. The IC₅₀ values ranged from 0.55- 5.91 μM. The cytotoxic screening showed that compounds 12c, 12g, 12b and 12a were found to have good cytotoxic activity with IC₅₀ of 0.55, 0.62, 0.73 & 0.80 μM respectively.

DNA fragmentation study

The compounds 12c and 12g which exhibited the lowest IC₅₀ values were selected for DNA fragmentation analysis to study the mechanism of cell death.

In compound 12g, DNA was incubated with three different concentration of the drug (1 μM, 6 μM & 10 μM). The control DNA showed no migration in the gel as there was no fragmentation of DNA. It formed a smear around the well. DNA treated with 1 μM concentration of drug also failed to induce fragmentation as shown in Figure 2a. This is understood from the fact that the DNA has migrated as a smear around the well. It was similar to the control sample. However, DNA samples treated with 6 μM and 10 μM of 12g showed fragmentation of DNA similar to the apoptotic ladder. DNA has initially moved as a smear and then shown fragmentation patterns similar to the ladder. As the concentration of 12g is increased from 6 μM to 10 μM, the extent of fragmentation has increased.

In case of Compound 12c, DNA sample was incubated with two different concentrations of the drug (3 μM & 4 μM). Compound 12c showed fragmentation of DNA similar to the standard ladder. At the concentration of 3 μM (Figure 2b), the fragmentation pattern showed the typical features of apoptotic DNA ladders in agarose gel. Compound 12c showed a better fragmentation profile as compared to 12g. This is because in 12g, DNA initially moved as a smear and then showed fragmentation. However, in 12c, the fragmentation of DNA was similar to the apoptotic ladder. Compound 12c shows uniform fragmentation at both 3 and 4 μM.

From these studies, we can conclude that compounds 12c and 12g induces DNA damage by apoptosis which probably is the biochemical basis for the cytotoxicity of these compounds.

CONCLUSION

We have synthesized and tested some novel 3-(4,5-substituted)-4H-1,2,4-triazol-3-ylthio)-N-isopropylpropan-1-amine derivatives for their cytotoxic activity. All compounds induced significant cytotoxic activity. Four compounds showed promising anti-inflammatory activity while the other five compounds showed moderate cytotoxic activity. The ability of compounds to induce cytotoxicity was confirmed by DNA fragmentation studies which indicated that cell death occurred by apoptosis. Further studies have to be carried out to explore the mechanistic basis of the cytotoxicity of these compounds.

ACKNOWLEDGMENTS

Authors are thankful to The Principal, JSS College of Pharmacy, Mysore, India for providing necessary facilities. Authors are beholden to DBT for the financial assistance (Ref. No:

BT/PR5594/MED/29/540/2012).

CONFLICT OF INTEREST

Declared None

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