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

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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 programming was 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 aromatase 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-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.

<table>
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<tr>
<th>S. No.</th>
<th>Name</th>
<th>Total Score</th>
<th>Crash</th>
<th>Polar</th>
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<tr>
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<td>12c</td>
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<td>0.6132</td>
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<tr>
<td>2</td>
<td>12g</td>
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<td>5</td>
<td>12d</td>
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</table>
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.

The precipitate formed was washed with petroleum ether to obtain 3-(4-(4-chlorophenyl)-5-(phenoxymethyl)-4H-1,2,4-triazole-3-thioyl)-N-isopropylpropan-1-amine (12e) Yield 44%, mp: 247-252°C.

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EI-MS m/z (%) 450.19 (M +, 62); Anal. Calcd (%) for C 21H24Cl2N4OS: C, 57.22; H, 4.04; N, 13.09; Found: C, 57.13; H, 3.95; N, 13.07.

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

Yield 36%, mp: 219-222

1H NMR (CDCl 3): (ppm) 25.5, 30.1, 36.7, 45.4, 47.9, 68.7, 115.8, 124.1, 126.1, 127.7, 128.6, 129.9, 147.4, 149.7, 150.7, 159.5; EI -MS m/z (%) 416.20 (M +, 40); Anal. Calcd (%) for C 21H25ClN4OS: 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-fluorophenyl)-4H-1,2,4-triazol-3-ylthio)-N-isopropylpropan-1-amine (12e)

Yield 52%, mp: 211-214

EI-MS m/z (%) 400.26 (M +, 44); Anal. Calcd (%) for C 21H25FN4OS: C, 62.98; H, 6.29; N, 13.99; Found: C, 62.87; H, 6.35; N, 14.12.

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

Yield 49%, mp: 239-242

EI-MS m/z (%) 394.17 (M +, 35); Anal. Calcd (%) for C 21H24Cl2N4OS: C, 61.54; H, 3.66; N, 13.35; Found: C, 61.60; H, 3.69; N, 13.45.

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. 5μl 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 μM, 2 μM, 4 μM, 6 μM, 8 μM and 10 μM and incubated in a shaker water bath at 37°C for 3 hours. 20μl of sample was transferred to an appendor containing 2μl of gel loading dye. The first well was loaded with 10μl of 1 Kb ladder. The remaining wells were loaded with 10 μl 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 ¼ 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 Doc instrumention (Biorad).

The cytotoxicity 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 doc image of the activity is represented in Figure 2.

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 IC_{50} 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 IC_{50} of 0.55, 0.62, 0.73 & 0.80 μM respectively.

DNA fragmentation study

The compounds 12c and 12g which exhibited the lowest IC_{50} values were selected for DNA fragmentation analysis to study the mechanism of cell death.

In compound 12g, DNA was incubated with three different concentrations of the drug (1 μM, 6 μM & 10μM). 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 11b 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. 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.

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CONFLICT OF INTEREST

Declared None

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