Abstract

Caspase, or cysteine-dependent aspartate-directed proteases, belong to a extremely protected group of cysteine proteases that have a crucial role within the numerous phases of apoptosis. The derivative of 2-(3,5-dibromo-4-methoxyphenyl)-3(3-mercapto-5(pyridine-4-Yl)4H1,2,4-triazol-4-yl)-thiazolidine4-one (C3) described a high toxic efficacy in murine melanoma tumor (B16F10), human prostate tumor (LCCaP), and non-small cell lung tumor (H1299) by measured half maximal inhibitory concentration IC₅₀ values were 41 µg/ml, 54.11 µg/ml, and 109.9 µg/ml, respectively, which was the most significant cytotoxic towards (B16F10) cell line treated at (P<0.0001) for 24 hours. No significant cytotoxic effect were observed in human neuronal glioblastoma cell line (U138 MG) and testes cell lines (Tera-1) at P-value (0.650), by comparison with normal cell line. Furthermore, 1,2,4-Triazole derivative (C3) encouraged In-vitro increase in caspase-9 activity in (B16F10) tumor cell line. Derivative (C3) effect on the mechanism of apoptosis reveal a highly increased caspase-9 activity, which observed at 10 µg/ml concentrate in B16F10 cell line, IC₅₀ was at 5.264 µg/ml. A series of 1,2,4-Triazole derivatives (C2, C3) were screened for their In-vitro antioxidant properties, through hydrogen peroxide, Nitric oxide, and total antioxidant capacity. The highest activity was indicated during measured (IC₅₀, TAC₅₀) values, with derivatives C2 and C3 by comparison with ascorbic acid as standard. Triazole derivative (C3) did not exhibit cytotoxic activity when applied human neuronal glioblastoma tumor (U138 MG) and testes cell line (Tera-1).

Keywords: Cell Viability, Statistical Analysis, Scavenging of Nitric oxide radical, Antioxidant Activity, caspase-9 activity assay.

1. Introduction

Caspase are endo proteases that breakdown amide bonds in an exceedingly reaction that based on chemical action cysteine residues within the caspase site and happens simply when sure aspartic acid residues within the substrate [1,2]. In spite of the very fact that caspase-mediated process will ends up in substrate inactivation, it would likewise produce active signal molecules that participate in ordered procedures [3]. The initiator caspases group includes of (caspase 2,8,9, and 10). The abettor pro-caspases exist as appropriate long pro-domains and monomers, that are activated by dimerization and not by cleavage [4]. From the literature survey it absolutely was found that one, 2,4-triazoles and their derivatives have grate importance healthful chemist and may be utilized to assembly diverse “heterocyclic” complexes together totally different biology efficacy like antiviral, anti-bacterial, anti-fungal, anti-tuberculosis, anticonvulsant drug, antidepressant drug, anti-inflammatory and anticancer efficacy [5]. Restraint of “glycogen synthase kinase3”, adversary of “GABA” senses, agonist’s of muscarinic pickup, be neuroleptic agent [6], and these compounds additionally show “anti-HIV-1”, toxic, antihistamine, also anti-proliferative phase performance [7]. The appearance elevation inspection framework has allowed us to esteem an outsized variety of tiny molecules in parallel and automatic fashions. In response to the present screening innovation, one in every of the best issues in recent drug discovery approach has been steered across a way planning and prepare complex bookstores obtaining in numerous biology protocols [8].

Inspect the effects of triazole synthetic on cell viability of the murine melanoma tumor B16F10, prostate tumor LCCaP, non-small...
cell lung tumor (H1299), neuronal glioblastoma tumor (U138 MG), and testes cell line (Tera-1), and apoptosis mechanism in B16F10 cell cycle phases by caspase-9 activity technique.

2. Materials and Methods
2.1. Chemistry
Melting points of the Triazole derivatives were determined using the microcontroller based melting point apparatus. It was recorded using KBr pellets in FTIR spectrometer (shimadzu 8700). 1H-NMR (400MHZ) spectra was recorded on a Varian spectrometer in CDCl3 solvent.

2.1.1. Synthesis of 4-[(3,5-dibromo-4-methoxybenzenylidene)-amino]-5(pyridine-4yl)-4H 1,2,4-triazole-3thiol (C2)
4-amino 5(pyridine-4-yl)-4H1,2,4-triazole-3thiol (0.03 mole) with aromatic aldehyde (0.03) was refluxed in absolute ethanol (50 ml) in a little drops of glacial aceticacid from 4 -7 hrs. The reflection blend was cool down, residue was filtered and recrystallized from ethanol [5].

2.1.2. Synthesis of 3-(3,5-dibromo-4-methoxyphenyl)-3(3-mercapto-5(pyridine-4yl)-4H 1,2,4- triazol-4yl) thiazolidine 4-one (C3)
A mixture of derivative (C2) (0.06 mole) and “Mercapto Acetic Acid” in (0.09 moles) in dry benzene (40 ml) was refluxed for 14 hours. The blend was intensified and recrystallized by applied ethanol [6].

![Synthetic pathways for the preparation of 2-(3,5-dibromo-4-methoxyphenyl)3(3mercapto-5(pyridine-4yl)4H1,2,4-triazol-4-yl)thiazolidin-4-one (C3).](image-url)
2.2. In Vitro cytotoxic activity of 1,2,4-Triazole derivative (C3)

Tumor cells in research inclusive the murine melanoma tumor B16F10, prostate tumor LCCaP, non-small cell lung tumor (H1299), neuronal glioblastoma tumor (U138 MG), and testes cell line (Tera-1).

2.2.1. Cell Viability

Cytotoxic of tumors (B16F10, LCCaP, H1299, U138 MG, Tera-1) was particular by “MTT” (3-[4,5-dimeathylthiazole-2-yl]-2,5-diphenyl tetraezolium broimide) protocol [9]. “MTT” protocol was instituted on the eclectic capacity living cells to decrease the yellow soluble tetraezolium corn, to a purple blue unsolvable “formazan” sediment. Residues of viability cell were obtained from 3 independent tests for each tumor type. Tumor cells were plated (1x10⁴ cells/well) in 96-well plates, these were brooded at 37°C, 10% CO₂ for 24hr. Next incubation period, agar was extracted and two –folded serial dilution the 1,2,4-Triazole derivative (C3) (600, 300, 150, 75, 37.5 µg/ml) were added to the plates. Three times were used for each concentration as well as the controls (normal plates). Plates were incubated at 37°C, 10% CO₂ for selected exposure time, 10µl of the “MTT” solution was added to each well. Agars were carefully removed and 120 µl for DMSO solubility solution was added per each well for 10 minutes, determine absorb by an “ELISA” reader at a wavelengths 570nm.

2.2.2. Scavenging of Nitric oxide radical

Scavenging of NO complete with O atom leading to decrease production of NO. The reaction mixture (10ml) containing sodium nitroprusside (20mM), phosphate buffer saline (PBS, pH 7.2, 2ml) and 1,2,4-Triazole derivatives (C2, C3) or standard (2ml) in DMSO at various concentrations was incubated at 25°C for 140 minutes. Next incubation period, 1.6 ml of the reflection blend containing nitrite ion was extracted, 2ml of sulpha nilicacid reagent was emphasis added, blended well and pliable to stand 10 minutes for dizatization completion reflection. Then 2ml of “NEDD” was emphasis added, mixed and pliable to stand at 30 minutes in prevalent light. A pink colored chromophore was created; absorbance was appearing in 540 nm [10].

2.2.3. Caspase –Glo 9 luminescent assay kit

Purified caspase-9 was diluted in (10 Mm) HEPES buffer (PH 7.2) with 0.1% prionex stabilizer and examine in a total volume of 200µl per well in 96-well plate. Contents the caspase-Glo 9 assay buffer were transferred into the amber bottle containing “caspase-Glo 9 substrate”, blend by rounding the contents until the substrate is totally fluxed to shape it. The reagent was allowed to equilibrate to room temperature, mix thoroughly after adding MG/132 inhibitor. These plates containing cells were removed from the incubator, 100 µl of caspase-9 reagent grade was added to each well of a “white-walled 96- well plate” containing 100 µl of gab, these plate were wrapped with a plate sealer. Contents well were blended softly using a plate shaker instrument about (300 rpm) for 35 seconds and incubated for 35 minutes. The luminescence was measured of each derivative in a plate/reading luminometer instrument [11].

2.3. Statistical Analysis Measurement

A one-way dissection of distinction ANOVA “Duncan” was completed to experience whether group distinction was considerable (p< 0.01) or not. The statistical analysis measurement “system- SAS” (2012) platform was utilized to study the difference factors in study parameters. Least significant difference-LSD experience was utilized for considerable compared among intermediary in this research [12].

3. Results and Discussion

3.1. Chemistry

FTIR (KBr, cm-1) for compound (C1): stretching bands of NH2 symmetric and asymmetrical at (3470, 3310), SH band at (2580). Bands at (3120.6) for (NH), bands of (C=N) and (C=C) aromatic appear at (1650.5) and (1540), respectively. 1H-NMR (CDCl3) (ppm): shows a singles at (7.231- 7.644) due to phenyl, single indicative at (6.562) for NH2, single indicative at 14.372 for (S-H) band.

FTIR (KBr, cm-1) for derivative (C2): disappearance of NH2 band and appearance of absorption bands at (1610-1627) for the imine (C=N) as evidence for
formation of derivative (C2). 1H-NMR (CDCl3) σ(ppm): appearance of singlet signal at 9.853 ppm for azomethine group (C=N), double signal at 8.3-8.8 for two hydrogen in pyridine ring, singlet signal at 12.951 for S-H bond.

FTIR (KBr, cm⁻¹) for derivative (C3): disappearance of (C=N) band and appearance of the carbonyl band due to thiazolidinone ring at (1725-1733) and the (C-S-C) band at (650-690 cm⁻¹). 1H-NMR (CDCl3) σ (ppm): single indicative at 3.24 due to methylene group (COCH2S), single indicative at 5.629 for CH(SCHN), double indicative of two hydrogen in pyridine ring at (7.9-8.4), triple signal for three hydrogen in methoxy group at 3.3, singlet signal at 13.785 for (S-H) group.

3.2. Cell Viability Measurement

From the results of (Table 1) it was found that 1,2,4-Triazole derivative (C3) having methylene group (COCH2S) with CH(SCHN) (Thiazoliedinone ring) displayed arise efficiency versus B16F10 cell line and LCCaP cell line together IC₅₀ values (41 µg/ml and 54.11 µg/ml) than the normal cell with IC₅₀ values (159.6 µg/ml and 150.5 µg/ml). Also, it was found that derivative (C3) exhibited a remarkable activity against H1299 cell line with IC₅₀ value (109.9 µg/ml) in significant difference at P-value (0.005) [8]. In addition, compound (C3) revealed no activity against U138 MG cell line and Tera-1 cell line, that no significant difference in P-value 0.650.

Table (1)

<table>
<thead>
<tr>
<th>Cell Lines</th>
<th>Triazole (C3) IC₅₀ µg/ml</th>
<th>Normal Cells IC₅₀ µg/ml</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>B16F10</td>
<td>41±2.2</td>
<td>159.6±8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LCCaP</td>
<td>54.11±1.4</td>
<td>150.5±0.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>H1299</td>
<td>109.9±0.8</td>
<td>205±0.51</td>
<td>0.005</td>
</tr>
<tr>
<td>U138 MG</td>
<td>150.2±2.9</td>
<td>172.6±2.9</td>
<td>0.650</td>
</tr>
<tr>
<td>Tera-1</td>
<td>125.4±5.2</td>
<td>149.7±4.6</td>
<td>0.650</td>
</tr>
</tbody>
</table>

1,2,4-Triazole derivative (C3) was estimated for their in-vitro toxic efficiency in respect diverse of cell lines after 24 hour of nursery using the criterion “MTT” protocol. According to the IC₅₀ results show in Fig.(2), Triazole derivative (C3) was obviously high powerful in the tumor plates and, thus, was used in moreover in-vitro researches. For murine melanoma cell lines (B16F10), derivative (C3) pretend have a minimize IC₅₀, however, this result was statistically considerable at (P<0.0001) by comparison with normal cells. Triazole derivative (C3) did not revelation cytotoxic efficiency when utilized human neuronal glioblastoma tumor (U138 MG) and testes cell line (Tera-1) [13], as shown in Fig.(3).
The pictures for “B16F10” tumor that have been treated together 1,2,4 triazole derivative (C3) as a positive control, and culture media (Normal cell) as a negative control, for 24hour. The cells were spotted with “Hoechst 33342 dye” (excitation 330/emission 420) which enables observation of cell loss, cell membrane permeability dye (excitation 491/emission 509), “MMP dye” (excitation 552/emission 576) for mitochondrial membrane potential changes, and goat anti-mouse secondary antibody conjugated with DyLight “TM 649” for cytochrome c emission. 1,2,4 triazole derivative (C3) spotted “B16F10” tumor and the spotted effect was not similar as for normal cell, as are shown in Fig.(4) below.

3.3. Antioxidant Activity

Derivative (C3) possessed potent inhibitory activity against hydrogen peroxide and total antioxidant capacity. A lower TAC and IC values indicates greater antioxidant activity with TAC value 784 µg/ml and TAC50 value 18.57 µg/ml, as shown in Table (2).
Table (2)

hydrogen peroxide and total antioxidant capacity of derivative (C3) in vitro.

<table>
<thead>
<tr>
<th>Derivative No.</th>
<th>IC(_{50}) ±SD (µg/ml)</th>
<th>Hydrogen peroxide</th>
<th>Nitric oxide</th>
<th>TAC</th>
<th>TAC(_{50})</th>
</tr>
</thead>
<tbody>
<tr>
<td>C2</td>
<td>44±0.38</td>
<td>46±0.01</td>
<td>782.2±0.48</td>
<td>18.57±0.36</td>
<td></td>
</tr>
<tr>
<td>C3</td>
<td>40±0.03</td>
<td>39±0.06</td>
<td>424±0.22</td>
<td>38.93±0.12</td>
<td></td>
</tr>
<tr>
<td>Ascorbic Acid</td>
<td>58±0.46</td>
<td>55±0.08</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

3.4. In vitro caspase-9 activity assay

Caspase-9 is effectiveness caspase and belongs to intrinsic apoptosis activation pathways [14]. The results of caspase-9 activity reveal that there is a significant increase in mean activity which was (16822, 108284) in B16F10 cells treated with 600 µg/ml and 150 µg/ml of 1,2,4-Triazole derivative (C3), respectively, at IC\(_{50}\) (5.264 µg/ml), as shown in Table (3). The caspase-9 activity technique are directly correlated to the toxic effect of derivative (C3) tested because it at these concentrations has an influence on cell membranes correlating with rapid variation in membrane permeability, which leads to reduced viability and cells death [15].

Table (3)

1,2,4-Triazole derivative (C3) effect of caspase-9 activity in murine melanoma cell line (B16F10) after incubation for 24 hours at 37\(^\circ\)C.

<table>
<thead>
<tr>
<th>Derivative (C3) concentration (µg/ml)</th>
<th>Caspase (Mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>600</td>
<td>16822±6252(^{a})</td>
</tr>
<tr>
<td>150</td>
<td>108284±3450(^{b})</td>
</tr>
<tr>
<td>75</td>
<td>33741±2960(^{c})</td>
</tr>
<tr>
<td>37.5</td>
<td>22564±2110(^{d})</td>
</tr>
<tr>
<td>0 µg/ml DMSO</td>
<td>4612±843.2(^{e})</td>
</tr>
<tr>
<td>LSD value</td>
<td>1180</td>
</tr>
</tbody>
</table>

Letters \(^{a,b,c,d}\) refers to significant result at P< 0.05
LSD = Least significant Difference

4. Conclusion

1,2,4-Triazole derivative (C3) was synthesized and this in vitro antitumor cytotoxic activity was evaluated on five tumor cell lines, which showed higher activity in B16F10 cell line. Derivatives (C2 and C3) can or may be used as easily accessible source of antioxidant and tumor treatment. Cytotoxic effect of 1,2,4-Triazole derivative (C3) has been demonstrated on B16F10 cell line by using caspase-9 activity assay at IC\(_{50}\) concentration measured.

Acknowledgements

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References

[6] Bhat S., Poojary D., Prasad P., Naik S., Synthesis and antitumor activity studies of some new fused 1,2,4-triazole derivatives


