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RESEARCH ARTICLE

3,4,5-Trisubstituted-1,2,4-triazole Derivatives as Antiproliferative Agents: Synthesis, *In vitro* Evaluation and Molecular Modelling

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Abstract: ***Background*:** Cancer is the name given to various diseases that are mainly uncontrolled, related to cell growth and can affect various organs. Among them, lung cancer is the one, which, in its earliest stages, is difficult to diagnose, and it is asymptomatic until the disease progresses. Triazole ring is an important heterocyclic ring known with various pharmacological activities.

***Objective*:** It is aimed to synthesize and characterize novel 1,2,4-triazole derivatives and screen them for *in vitro* antiproliferative activity and binding analysis through docking studies.

***Method*:** In this study, we have synthesized new 2-[[5-[(4-aminophenoxy)methyl]-4-phenyl-4*H*-1,2,4-triazol-3-yl]thio]-*N*-(substituted aryl)acetamide (**5a-h**) derivatives and investigated their anticancer activities against human lung cancer (A549) and mouse embryo fibroblast cell lines (NIH/3T3) by MTT, flow cytometric, caspase-3 and matrix metalloproteinase-9 (MMP-9) inhibition assays.

***Results*:** Compounds **5f, 5g** and **5h** showed the highest cytotoxicity and caused significant apoptosis. These compounds inhibited MMP-9, slightly whereas they did not effect caspase-3.

***Conclusion*:** **5f** namely, *N*-(5-acetyl-4-methylthiazol-2-yl)-2-((5-((4-aminophenoxy)methyl)-4-phenyl-4*H*-1,2,4-triazol-3-yl)thio)acetamide exhibited as the most active compound with selective cytotoxicity and the highest MMP-9 inhibition. Besides, molecular modelling assessment was signified that antiproliferative activity of the compounds **5f, 5g** and **5h** was through a slight MMP-9 inhibition pathway.

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1. INTRODUCTION

Today, cancer is the most deadly disease due to high mortality rates and challenging treatment process, including surgical operations, intense chemotherapy and/or radiotherapy. Besides, conditions such as decreased quality of life and recurrence of the disease increase the difficulties [1, 2]. The biochemical processes involving signals and enzymes have been identified as responsible for tumor formation and progression in many different types of cancer, therefore, new drug synthesis studies are aimed to suppress these pathways and thus ensure stability or cure the disease completely. Current approaches in the exploration of novel anticancer therapies are directed towards the discovery of appropriate molecules that act as specific inhibitors of key enzymes, which play an important role in carcinogenesis [3].

Apoptosis is a natural process in which cells give their own termination signals and is crucial for normal tissue regeneration and development. Cancer cells stop this period and proliferate uncontrollably and cause tumor formation. Mechanistically blocking key proteins and/or enzymes responsible for apoptosis is an up-to-date approach for anticancer chemotherapy [4, 5]. Caspases are intracellular cysteine proteases, which mediate apoptosis and provide maintaining homeostasis through regulating cell death, inflammation [6]. Furthermore, Matrix Metalloproteinase (MMP) enzymes are the main mediators of alterations on the microenvironment of cancer cells that their inhibitors act as apoptotic inducers to prevent invasion and metastasis of tumors [7]. In anticancer treatment and new drug discoveries, it is desirable to achieve more powerful,

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effective drugs with reduced side-effects, in particular targeting the mitochondrial pathway [8].

1,2,4-Triazole ring is a versatile lead molecule, which has a wide spectrum of biological activity [9-11]. The triazole derivatives possess chemical properties like moderate dipole character, hydrogen bonding capability, rigidity and stability, providing convenient drug-receptor/enzyme interactions [12, 13]. Many triazole derivatives have been reported with anticancer activity [14-17]. Among them, 5-mercapto-1,2,4-triazoles have been frequently observed as anticancer agents [18, 19]. Generally, 1,2,4-triazole derivatives have been determined to exhibit anticancer activity as kinase inhibitors, tubulin modulators, methionine aminopeptidase, tankyrase, carbonic anhydrase, aromatase and sulfatase inhibitors [20-22]. Furthermore, some derivatives were detected to show activity based on caspase [23] and matrix metalloproteinase [24] inhibitory activities that led tumor cells to apoptosis.

In addition, thiazole ring has become a prominent and preferred ring system in new drug discovery studies due to its ease of synthesis and existing in many drug structures. In particular, it has been found in the structure of anticancer drugs. Bleomycine, sulfathiazole, thiazofurine, dasatinib and its derivatives make the skeleton more potent and safer for molecules where it is incorporated [25, 26].

In this review, we have studied novel triazole derivatives bearing aryl/thiazole rings through molecular hybridization, determined their antiproliferative and apoptotic activity on A549 cell line and evaluated inhibition potency on caspase and MMP-9 enzymes.

2. MATERIALS AND METHODS

2.1. EXPERIMENTAL

All chemicals used in the syntheses were purchased either from Merck Chemicals (Merck KGaA, Darmstadt, Germany) or Sigma-Aldrich Chemicals (Sigma-Aldrich Corp., St. Louis, MO, USA). The reactions and the purities of the compounds were observed by Thin-Layer Chromatography (TLC) on silica gel 60 F254 aluminum sheets obtained from Merck (Darmstadt, Germany). Melting points of the synthesized compounds were recorded by the MP90 digital melting point apparatus (Mettler Toledo, Ohio, USA) and were presented as uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded by a Bruker 300 MHz and 75 MHz digital FT-NMR spectrometer (Bruker Bioscience, Billerica, MA, USA) in DMSO-d_6, respectively. In the NMR spectra, splitting patterns were designated as follows: s: singlet; d: doublet; t: triplet; m: multiplet (Supplementary material). Coupling constants (*J*) were reported as Hertz. High resolution mass spectrometric (HRMS) studies were performed using an LC/MS-IT-TOF system (Shimadzu, Kyoto, Japan). Elemental analyses were performed on a Leco 932 CHNS analyzer (Leco, Michigan, USA).

2.1.1. Ethyl 2-(4-acetamidophenoxy)Acetate (1)

N-(4-hydroxyphenyl)acetamide (0.033 mol, 5g), ethyl chloroacetate (0.04 mol, 4.88 g) and potassium carbonate (0.033 mol, 4.55 g) were refluxed in 200 mL acetone for 6 h. After TLC, the solvent was evaporated, and the material was treated with water and filtered, then crystallized from ethanol.

2.1.2. N-(4-(2-hydrazinyl-2-oxoethoxy)phenyl)Acetamide (2)

Ethyl 2-(4-acetamidophenoxy)acetate (**1**) (0.028 mol, 6.64 g) and 0.08 mol (4 mL) of 85% hydrazine monohydrate were stirred in ethanol (250 mL) at room temperature overnight. The reaction was controlled by TLC and the mixture was allowed to separate from the solvent. The precipitated material was filtered and washed with excess ethanol.

2.1.3. N-(4-(2-oxo-2-(2-(phenylcarbamothioyl)hydrazineyl) ethoxy)phenyl)Acetamide (3)

N-(4-(2-hydrazinyl-2-oxoethoxy)phenyl)acetamide (**2**) (0.02 mol, 4.78 g) and phenyl isothiocyanate (0.022 mol, 2.6 mL) were refluxed in 250 mL ethanol for 3 h. The reaction was controlled by TLC and terminated. The solvent was evaporated and the raw product was acquired.

2.1.4. 3-(4-Aminophenyl)-5-mercapto-4-phenyl-4H-1,2,4-triazol (4)

N-(4-(2-oxo-2-(2-(phenylcarbamothioyl)hydrazinyl)ethoxy)phenyl)acetamide (**3**) (0.011 mol, 4.11 g) was refluxed with 80 mL 2N potassium hydroxide prepared in ethanol. After TLC, the pH was adjusted to 7 in a cold environment to precipitate the material, then the product was taken by filtration and recrystallized from ethanol.

2.2. General Procedure for the Synthesis N-substituted-2-((5-((4-aminophenoxy)methyl)-4-phenyl-4H-1,2,4-triazol-3-yl)thio)Acetamide Derivatives (5a-5h)

N-(4-((5-mercapto-4-phenyl-4*H*-1,2,4-triazol-3-yl)methoxy)phenyl)acetamide (**4**) (0.0015 mol, 0.5 g) and equivalent mole of 2-chloro-*N*-substituted acetamide derivatives were reacted in the presence of potassium carbonate (0.0015 mol, 0.21 g) in acetone. From the reaction mixture, the solvent was evaporated and treated with water to yield the final product.

2.2.1. 2-((5-((4-aminophenoxy)methyl)-4-phenyl-4H-1,2,4-triazol-3-yl)thio)-N-phenylacetamide (5a)

Yield: 65%. m. p. 103°C, ^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ 4.21 (s, 2H, S-CH_2), 4.71 (s, 2H, NH_2), 4.91 (s, 2H, O- CH_2), 6.42-6.55 (m, 3H, Ar-H), 7.08 (s, 1H, Ar-H), 7.31-7.36 (m, 2H, Ar-H), 7.53-7.64 (m, 8H, Ar-H), 10.40 (brs, 1H, NH). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ 37.92 (S-CH_2), 61.92 (O-CH_2), 115.65, 117.28, 120.16, 120.74, 124.62, 128.08, 129.92, 130.86, 131.15, 133.80, 139.88, 144.52, 149.68, 152.46, 153.24, 166.51. For $C_{23}H_{21}N_5O_2S$ calculated: 64.02 % C, 4.91 % H, 16.23 % N; found: 63.92 % C, 4.93 % H, 16.18 % N. HRMS (m/z): $[M+2H]/2^+$ calculated for $C_{23}H_{21}N_5O_2S$: 216.5781; found: 216.5789.

2.2.2. 2-((5-((4-aminophenoxy)methyl)-4-phenyl-4H-1,2,4-triazol-3-yl)thio)-N-(4-chlorophenyl)Acetamide (5b)

Yield: 72%. m. p. 127°C, ^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ 4.18 (s, 2H, S-CH_2), 4.68 (s, 2H, NH_2), 4.89 (s,

2H, O- CH_2), 6.39-6.53 (m, 4H, Ar-H), 7.36-7.60 (m, 9H, Ar-H), 10.51 (brs, 1H, NH). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ 37.22 (S-CH_2), 61.29 (O-CH_2), 115.02, 116.65, 121.08, 127.44, 129.22, 130.24, 133.15, 138.20, 143.89, 149.03, 151.76, 152.63, 166.08. For $C_{23}H_{20}ClN_5O_2S$ calculated: 59.29% C, 4.33% H, 15.03% N; found: 59.28% C, 4.32% H, 15.05% N, 6.88. HRMS (m/z): $[M+2H]/2^+$ calculated: 233.5586; found: 233.5593.

2.2.3. 2-((5-((4-aminophenoxy)methyl)-4-phenyl-4H-1,2,4-triazol-3-yl)thio)-N-(4-methoxyphenyl)Acetamide (5c)

Yield: 70%. m. p. 178°C, ^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ 3.70 (s, 3H, O-CH_3), 4.15 (s, 2H, S-CH_2), 4.68 (s, 2H, NH_2), 4.89 (s, 2H, O-CH_2), 6.39-6.43 (m, 2H, Ar-H), 6.50-6.53 (m, 2H, Ar-H), 6.87-6.90 (d, J:9.12 Hz, 2H, Ar-H), 7.44-7.59 (m,7H, Ar-H), 10.22 (brs, 1H, NH). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ 37.21 (S-CH_2), 55.61 (O-CH_3), 61.29 (O-CH_2), 114.36, 115.01, 116.65, 121.08, 127.45, 130.22, 130.50, 132.37, 133.18, 143.89, 149.04, 151.85, 152.59, 155.80, 165.31. For $C_{24}H_{23}N_5O_3S$ calculated: 62.46% C, 5.02% H, 15.17% N; found: 62.52% C, 5.03% H, 15.07% N. HRMS (m/z): $[M+2H]/2^+$ calculated: 231.5834; found: 231.5837.

2.2.4. 2-((5-((4-aminophenoxy)methyl)-4-phenyl-4H-1,2,4-triazol-3-yl)thio)-N-(4,5-dimethylthiazol-2-yl)Acetamide (5d)

Yield: 75%. m. p. 206°C, ^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ 2.15 (s, 3H, thiazole- CH_3), 2.22 (s,3H, thiazole-CH_3), 4.18 (s, 2H, S-CH_2), 4.68 (s, 2H, NH_2), 4.88 (s, 2H, O-CH_2), 6.39-6.41 (m, 2H, Ar-H), 6.49-6.54 (m, 2H, Ar-H), 7.50-7.56 (m, 5H, Ar-H), 12.20 (brs, 1H, NH). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ 10.84 (thiazole- CH_3), 14.76 (thiazole- CH_3) 35.87 (S-CH_2), 61.30 (O-CH_2), 115.01 115.77, 116.63, 119.30, 120.89, 127.44, 130.23, 130.53, 133.13, 143.89, 149.06, 152.70, 165.89. For $C_{22}H_{22}N_6O_2S_2$ calculated: 56.63% C, 4.75% H, 18.01% N; found: 56.64% C, 4.79% H, 17.59% N. HRMS (m/z): $[M+2H]/2^+$ calculated $C_{23}H_{20}ClN_5O_2S$: 234.0696; found: 234.0698.

2.2.5. 2-((5-((4-aminophenoxy)methyl)-4-phenyl-4H-1,2,4-triazol-3-yl)thio)-N-(5-phenylthiazol-2-yl)Acetamide (5e)

Yield: 68%. m. p. 180°C, ^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ 4.21 (s, 2H, S-CH_2), 4.68 (s, 2H, NH_2), 4.89 (s, 2H, O- CH_2), 6.39-6.42 (m, 2H, Ar-H), 6.49-6.54 (m, 2H, Ar-H), 7.28-7.33 (m, 1H, Ar-H), 7.42 (t, J:7.39 Hz, 2H, Ar-H), 7.49-7.61 (m, 6H, Ar-H), 7.89 (d, J:8.28Hz, 2H, Ar-H). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ 36.73 (S-CH_2), 55.61 (O-CH_3), 61.30 (O-CH_2), 108.13, 115.02, 115.79, 116.64, 120.89, 126.08, 127.47, 128.04, 129.14, 130.23, 130.50, 133.21, 135.06, 143.88, 149.07, 151.92, 152.59, 167.31. For $C_{26}H_{22}N_6O_2S_2$ calculated: 60.68% C, 4.31% H, 16.33% N; found: 60.69% C, 4.29% H, 16.31% N. HRMS (m/z): $[M+2H]/2^+$ calculated: 234.0696; found: 234.0693.

2.2.6. N-(5-acetyl-4-methylthiazol-2-yl)-2-((5-((4-aminophenoxy)methyl)-4-phenyl-4H-1,2,4-triazol-3-yl)thio)Acetamide (5f)

Yield: 69%. m. p. 193°C, ^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ 2.32 (s, 3H, thiazole-CH_3), 2.40 (s, 3H, acetyl-CH_3), 3.36 (s, 2H, NH_2), 4.25 (s, 2H, S-CH_2), 4.89 (s, 2H, O-CH_2), 6.39-6.42 (m, 2H, Ar-H), 6.49-6.52 (m, 2H, Ar-H), 7.48- 7.51 (m, 2H, Ar-H), 7.54-7.89 (m, 3H, Ar-H), 7.84 (s, 2H, Ar-H). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ 18.78 (thiazole-CH_3), 29.91 (acethyl-CH_3) 35.85 (S-CH_2), 61.27 (O-CH_2), 115.01, 116.62, 126.13, 127.41, 129.24, 130.25, 130.57, 133.08, 143.89, 149.05, 151.36, 152.76, 154.82, 158.17, 159.79, 167.37 (C=O), 170.96, 188.71, 191.19 (acetyl C=O). For $C_{23}H_{22}N_6O_3S_2$ calculated: 55.86% C, 4.48% H, 16.99% N; found: 55.85% C, 4.50% H, 16.92% N. HRMS (m/z): $[M+2H]/2^+$ calculated: 248.0670; found: 248.0667.

2.2.7. Ethyl 2-(2-((5-((4-aminophenoxy)methyl)-4-phenyl-4H-1,2,4-triazol-3-yl)thio)acetamido)-5-methylthiazole-4-carboxylate (5g)

Yield: 77%. m. p. 216°C, ^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ 1.27 (t, J:9.51 Hz, 3H, CH_2-$\underline{CH_3}$), 2.55 (s, 3H, thiazole -CH_3), 3.35 (brs, 2H, NH_2), 4.20-4.26 (m, 4H, S-CH_2 and $\underline{CH_2}$-CH_3), 4.89 (s, 2H, O-CH_2), 6.40 (d, J:9.51 Hz, 2H, Ar-H), 6.51 (d, J:9.51 Hz, 2H, Ar-H), 7.49-7.58 (m, 5H, Ar-H). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ 14.64 (CH_2-$\underline{CH_3}$), 17.51 (thiazole-CH_3), 35.89 (S-CH_2), 61.02 (O-$\underline{CH_2}$-CH_3), 61.28 (O-CH_2), 115.01, 116.62, 127.41, 130.25, 130.57, 133.08, 143.89, 149.05, 151.38, 152.75, 156.72, 160.01, 162.51 (carboxylate C=O), 167.47 (C=O). For $C_{24}H_{24}N_6O_4S_2$ calculated: 54.95% C, 4.61% H, 16.02% N; found: 54.97% C, 4.63% H, 16.00% N. HRMS (m/z): $[M+2H]/2^+$ calculated for $C_{24}H_{24}N_6O_4S_2$: 263.0723; found: 263.0728.

2.2.8. Ethyl 2-(2-((5-((4-aminophenoxy)methyl)-4-phenyl-4H-1,2,4-triazol-3-yl)thio)acetamido)-4-methylthiazole-5-carboxylate (5h)

Yield: 70%. m. p. 211°C, ^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ 1.71 (brs, 3H, O-CH_2-$\underline{CH_3}$), 2.97 (brs, 3H, thiazole- CH_3), 3.80 (brs, 2H, NH_2), 4.67-4.69 (s, 4H, S-CH_2 and O-$\underline{CH_2}$-CH_3), 5.33 (s, 2H, O- CH_2), 6.85 (d, J:7.42 Hz, 2H, Ar-H), 6.95 (d, J:7.42 Hz, 2H, Ar-H), 7.95-8.01 (m, 5H, Ar-H). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ 15.08 (CH_2-$\underline{CH_3}$), 17.93 (thiazole-CH_3), 36.25 (S-CH_2), 61.49 (O-$\underline{CH_2}$-CH_3), 61.71 (O-CH_2), 115.14, 117.06, 127.85, 130.69, 131.02, 133.52, 144.32, 149.50, 151.78, 153.21, 157.14, 160.23, 162.94 (carboxylate C=O), 167.83 (C=O). For $C_{24}H_{24}N_6O_4S_2$ calculated: 54.95% C, 4.61% H, 16.02% N; found: 54.94% C, 4.60% H, 16.01% N. HRMS (m/z): $[M+2H]/2^+$ calculated: 263.0723; found: 263.0718.

3. PHARMACOLOGY

3.1. Cytotoxicity Test

NIH/3T3 Mouse embryonic fibroblast (ATCC® CRL-1658™, American Type Culture Collection (ATCC), Manassas, VA, USA) and A549 human lung adenocarcinoma (ATCC® CCL-185™, American Type Culture Collection (ATCC), Manassas, VA, USA) cell lines were used for cytotoxicity tests. NIH/3T3 cell line was used to investigate the selectivity of the compounds, whereas other cell lines were used to determine anticancer activity. These cell lines were incubated according to the instructions

of the supplier at 37°C in a humidified atmosphere of 95% air and 5% CO_2. NIH/3T3 and A549 cells were seeded at 1x104 cells into each well of 96-well plate. After 24 h of incubating period, the culture mediums were removed and the compound was added to the culture medium at 500–3.9 μg/mL doses with a dilution factor of 2 (3.9, 7.8, 15.6, 31.2, 62.5, 125, 250, 500 μg/mL). After 24 h of incubation, MTT (3- (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was performed, as previously described [27]. Percentage of inhibition was calculated for each concentration of the compounds according to the formula below, obtained from the instructions of the manufacturer and IC_{50} values were estimated by plotting a dose-response curve of the inhibition% versus test compound concentrations. All experiments were done in quadruplicates at three different time points, and the results were given as Mean ± standard deviation. Cisplatin was used as a positive control. The stock solutions of the compounds were prepared in dimethyl sulfoxide (DMSO) and further dilutions were made with fresh culture medium. The final DMSO concentration was under 0.1%.

3.1.1. The Determination of Early/Late Apoptosis by Flow Cytometry

The A549 cells were seeded at 10^5 cells/mL per well in six-well plates at 37°C in a humidified atmosphere containing 5% CO_2 in air. Then, cells were treated with cisplatin and active compounds at IC_{50} doses for 24 h. The A549 cells were harvested and washed twice with ice-cold PBS and resuspended in 100 μL of binding buffer. A volume of 5 μL (5 μg/mL) of Annexin V-FITC and PI were added to the cells and incubated for 15 min in the dark at room temperature (20-25°C). Then, 400 μL of binding buffer was added to the mixture samples and analyzed with a flow cytometer (BD FACS Aria Cell Sorter flow cytometry, BD Biosciences) [28].

3.1.2. Spectrofluorometric Analysis of Caspase-3 Activation

Caspase-3 activation was analyzed by the Spectrofluorometric Caspase-3 Assay kit (BD Pharmingen, Franklin Lakes, NJ). Kit was designed to measure caspase-3 or DEVD-cleaving activity, an early marker of cells undergoing apoptosis. First, cells (1x106 cells/mL) were washed with Phosphate-Buffered Saline (PBS), resuspended in cold cell lysis buffer and incubated for 30 min on ice. After 24 h incubation period with various concentrations of compounds 5f, 5g, 5h and cisplatin, cell lysates were prepared. For each reaction, 5 mL of reconstituted AcDEVD-AMC (synthetic tetrapeptide fluorogenic substrate for Caspase-3 activity) was added to a well containing 0.2 mL of 1xHEPES buffer. Cell lysate (20 mL) was added to each well/ reaction. Reaction mixtures were incubated for 1 h at 37°C. The amount of AMC liberated from Ac-DEVD-AMC was measured using microplate reader (Perkin Elmer/Victor/X3) with an excitation wavelength of 380 nm and an emission wavelength of 460 nm. Apoptotic cell lysates containing active Caspase-3 yielded a considerable emission as compared to controls. In addition, non-apoptotic control cell lysates AMC emission was accepted as 100% and other cell lysates emissions were measured according to control cells emissions. All experiments were repeated twice. For all doses, duplicate wells were used.

3.1.3. Matrix Metalloproteinase-9 (MMP-9) Inhibition Assay

MMP-9, colorimetric kits were purchased from Enzo Life Sciences Inc. (Farmingdale, New York, NY, USA). The MMP Colorimetric Drug Discovery Kits are a complete assay system designed to screen MMP inhibitors using a thiopeptide as a chromogenic substrate (Ac-PLG-[2-mercapto-4-methyl-pentanoyl]-LG-OC_2H_5). The MMP cleavage site peptide bond is replaced by a thioester bond in the thiopeptide. Hydrolysis of this bond by an MMP produces a sulfhydryl group, which reacts with DTNB [5,50-dithiobis(2-nitrobenzoic acid), Ellman's reagent] to form 2-nitro-5-thiobenzoic acid, which can be detected by its absorbance at 412 nm. The assays were conducted in triplicate. The UV absorbance was read at 412 nm using a microplate reader (BioTek, PowerWave, Gen5 software, Winooski, VT, USA) at room temperature. NNGH was used as a control inhibitor. Data was expressed as Mean±SD. The inhibitor % remaining activity of MMPs was calculated using the following equation:

Inhibitor% activity remaining = (V inhibitor/V control) × 100.

The inhibition (percent) of MMPs was calculated using the following equation:

I (%) = 100 – Inhibitor % activity remaining

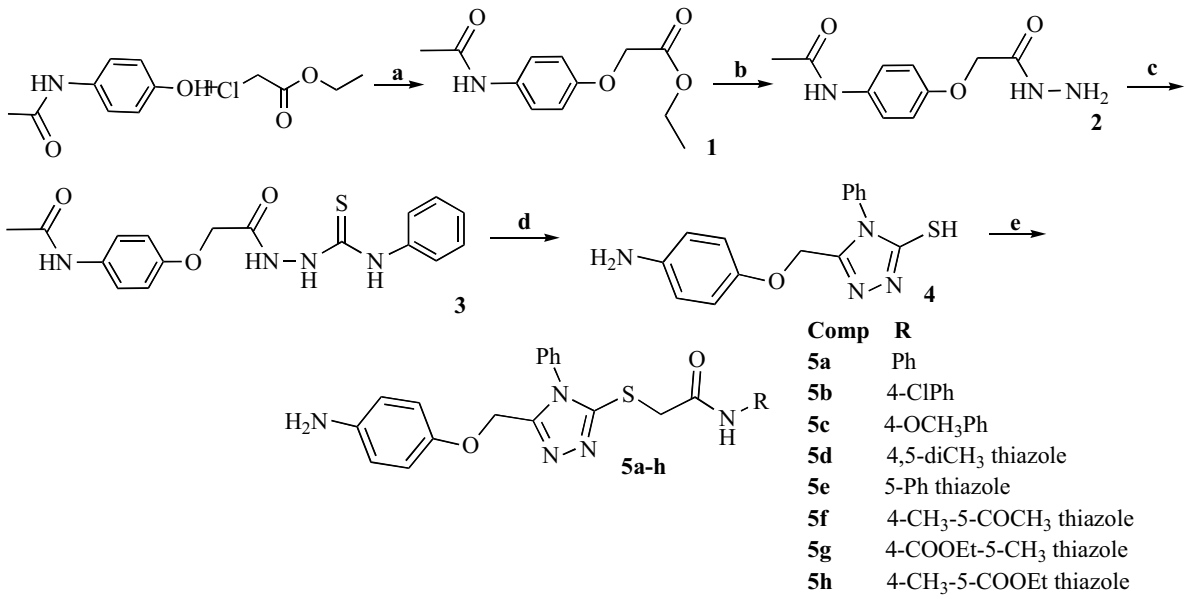
3.1.4. Docking Studies

Crystal structure of MMP-9 was retrieved from the Protein Data Bank server (PDB code: 5I12). Protein preparation process, ligand preparation process, grid generation, docking and visualization studies were worked on Schrodinger's Maestro molecular modeling package [29].

The water molecules were removed from the crystal structure. Ligands were set to the physiological pH (pH = 7.4) at the protonation step. In molecular docking simulations: Glide/XP docking protocols were applied for the prediction of topologies of 5f, 5g and 5h at the active site of target structure [30], then they were docked to the active site of 5I12.

4. RESULTS

In this work, novel 3,4,5-trisubstituted triazole derivatives were synthesized with a five-step synthetic procedure. First of all, *N*-(4-hydroxyphenyl)acetamide and ethyl chloroacetate were refluxed in acetone to acquire ester intermediate (**1**). The obtained product, ethyl 2-(4-acetamidophenoxy)acetate (**1**) was reacted with hydrazine monohydrate in ethanol, then hydrazinated compound **(2)** was reacted with phenyl isothiocyanate to synthesize *N*-(4-(2-oxo-2-(2-(phenylcarbamothioyl)hydrazineyl)ethoxy)phenyl)acetamide (**3**). In the next step, the mentioned intermediate was directed to a ring closure reaction through 2N potassium hydroxide. This reaction was terminated by controlling TLC and pH of the mixture was set to 7 by placing the material in a cold environment to provide triazole ring formation and acetyl group hydrolysis. All intermediates were obtained in good yields. At the last step, the resulted triazole molecule (**4**) was



Scheme 1. Synthesis of the compounds **5a-h.** Reagents and conditions: a) Acetone, K_2CO_3, 250°C, 6h; **b**) EtOH, $H_2NNH_2.H_2O$, rt; **c**) Phenyl isothiocyanate, EtOH, 250°C, 3h; **d**) 2N ethanolic-KOH, 300°C; **e**) 2-Chloro-*N*-aryl/heteroarylacetamides, Acetone, K_2CO_3, rt.

Table 1. Some properties of synthesized compounds.

-	Physicochemical Properties						MedChem
	MW	HBA	HBD	TPSA	Log $P_{o/w}$	Log S (Ali)	RoF (V)
5a	431.51	6	1	123.44	3.29	-5.79	Yes (0)
5b	465.96	5	2	123.44	3.86	-6.44	Yes (1)
5c	461.54	6	2	132.67	3.35	-5.95	Yes (1)
5d	466.58	6	2	161.10	3.00	-6.25	Yes (1)
5e	514.62	6	2	161.10	3.72	-7.15	Yes (1)
5f	494.59	7	2	178.17	2.68	-5.94	Yes (1)
5g	524.61	8	2	187.40	2.94	-6.89	No (2)
5h	524.61	8	2	187.40	2.75	-6.68	No (2)
SD-1	344.38	7	1	110.39	1.37	-3.76	Yes (0)
SD-2	300.05	7	1	6.48	1.37	-3.76	Yes (0)

MW: Molecular Weight, **HBA:** H-bond acceptor, **HBD:** H-bond acceptor, **TPSA:** Topologic polar surface area (Å²) **Log $P_{o/w}$:** *Consensus* Log $P_{o/w}$ (Average of all five predictions), **Log S:** Water Solubility, **GIA:** Gastrointestinal absorption, **Log K_p:** skin permeation **(cm/s) RoF (V)**: Rule of Five (violation number), **MedChem:** Medicinal Chemistry, **SD-1**: Standard Drug (*N*-Isobutyl-*N*-(4-methoxyphenylsulfonyl)glycyl hydroxamic acid), **SD-2**: Standard Drug (Cisplatin).

treated with appropriate 2-chloro-*N*-substituted acetamide derivatives to get the final eight molecules (**5a-h**) (Scheme **1**). The target compounds (**5a-h**) were obtained purely and the structures of the compounds were elucidated with spectroscopic methods.

The structure of the synthesized materials was elucidated by ^{1}H-NMR, ^{13}C-NMR, HRMS and elemental analysis. The ppm values of the peaks that belong to -NH, O-CH_2 and S-CH_2 protons were seen at 10.20-12.20 ppm and 4.88-5.51 ppm and 4.15-4.81 ppm, respectively in all compounds. The signal at 3.36 ppm obtained for **5c** was a distinctive peak for CH_3 substituent. The signals belong to CH_3 protons for compound **5d** were observed at 2.15 and 2.22 ppm. The expected peaks for CH_3 group in **5f** molecule were seen as singlets at 2.32 and 2.40 ppm. For compound **5g**, CH_3 and O-CH_2-CH_3 protons were seen at 2.55, 4.26 and 1.27 ppm, respectively. Compound **5h** also showed peaks for CH_3 and O-CH_2-CH_3 protons at 2.97 ppm, at 4.69 ppm, and 1.71 ppm. Additionally, multiple peaks were observed in the aromatic region due to the phenyl or thiazole rings. In the ^{13}C-NMR spectra of the compounds, signals belong to S-CH_2 and O-CH_2 were detected at 35.84-37.92 ppm and 61.02-61.92 ppm, respectively. HRMS of the compounds was processed

in positive ion mode and peaks were detected in accordance with half of the molecular weights of the compounds.

Some properties of synthesized compounds were displayed in Table **1**, which were calculated by SwissADME [31-33]. The number of HBA was calculated to be minimum 5 and maximum 8, and also the number of HBD was 2, except **5a**. Log P values were estimated between 2.68 and 3.86. All compounds have violations of RoF equal to or less than 2. Therefore, the final compounds may be applied for oral use according to forecast [34]. In fact, these findings were also in harmony with activity results. Especially, compound **5f** stand out *via* TPSA (178.17 Å²) and Log P (2.68). These differences between **5f** and other derivatives may be one of the reasons, which explain the incoherent in the activity tests due to the solubility.

5. PHARMACOLOGY

All compounds (**5a-5h)** and the standard drugs were evaluated for their anticancer activity and mechanism against A549 and NIH/3T3 cell lines. Results of IC_{50}, apoptosis, caspase-3 inhibition and MMP-9 inhibition were shown in Tables **2-5**, respectively.

5.1. 50% Inhibition Concentration and Selectivity Index (SI)

According to MTT results, compounds **5a, 5f, 5g** and **5h were** exhibited, but the rest of them did not display cytotoxicity against A549 cells. Moreover, **5a-5h** significantly killed NIH/3T3 cells. On the other side, **5a** and **5j** showed a low cytotoxic effect against A549. Compounds **5f**, **5g** and **5h** (IC_{50}: <3.90 for them) showed excellent activity against A549 cell line. Additionally, the selectivity index of compound **5f** was calculated greater than 100, which is an important property for anticancer drugs.

5.2. Apoptotic and Necrotic Cells

Tested compounds encouraged apoptosis more than cisplatin. Compound **5g** was better than **5h**, and **5h** was better than **5f**, and **5f** was better than cisplatin (Fig. **1**). In addition, necrotic cells values of **5g**, **5f**, **5h** and cisplatin were determined to be 26.0, 29.6, 33.9 and 53.9, respectively. Therefore, synthesized compounds could be preferable molecules in proportion to cisplatin. IC_{50} values and apoptotic-necrotic cells values together pointed to **5f**.

5.3. Enzyme Studies

After the determination of active compounds pursuant to induced apoptosis and reduced necrosis, their activation pathway was tested on pharmacological mechanisms, caspase-3 and MMP-9.

Entreated compounds displayed low positive cell percentages (**P4**) in the enzyme study, which clarified that the induced apoptosis rates were independent of caspase-3 activation. On the other hand, active compounds (**5f**, **5g** and **5h**), non-effective compound (**5d**) and standard drug (NNGH) were tested to determine MMP-9 inhibition activity. The findings were found meaningful with IC_{50} values. In accordance with the test results, the most active compound was determined as **5f**. These results were worthy of note that the development of new treatments against lung carcinoma, design and synthesis to new molecules to candidate anticancer drugs. Thus, our investigation was proceeded to the next step to elucidate structure-activity Relationship (SAR) with molecular docking study.

5.4. Molecular Docking and Structure-Activity Relationship (SAR) Studies

For comparing the active and non-active/low effective structures, **5d, 5f**, **5g** and **5h** were docked to the related enzyme (PDB ID: 5I12). The docking poses were collected, as shown in (Figs. **2-9**). According to docking results, compound **5f** interacted with Ala191, His226, Ala227 and Tyr245 active site amino acids and Zn301 metal ion. Between triazole and histamine, amino acid occurred π-π stacking, and three H-bonds were viewed with the nitrogen of acetamide and aniline. There was metal coordination between triazole's nitrogen (3^{rd} and 4^{th}) and Zn301. In addition, π -cation interaction has occurred between aniline and Zn301. Compound **5g** interacted with Ala191, His226, Glu227, His236 and Tyr245 amino acids and Zn301 metal ion. Between triazole and histamine, amino acids occurred π-π stacking. There were three H-bond displayed like compound **5h**. Also, Zn301 sympathized with triazole nitrogen (3^{rd}) as metal coordination and aniline as π-cation. Compound **5h** interacted with Gly186, Leu188, His226 and His236 amino acids and Zn301 metal ion. Between aromatic rings (thiazole and phenyl) and histamine amino acids occurred π-π stacking. There were two H-bonds, which were with Leu188 and Gly186. Zn301 built a salt bridge with acetamide nitrogen, and displayed π-*cation* interaction with phenyl ring.

The docking results point out that zinc ion and its bonding amino acid residues (His226, His230 and His336) are important for active compounds (**5f**, **5g** and **5h**) in MMP-9 inhibition activity. Previous studies mentioned that zinc ion at the active site of the catalytic domain is closely related to the hydrolytic activity of MMP families [30, 35]. Thus, the effectiveness depends not only on the hydrogen bond but also on the bonds that the ligand forms with histamine and zinc. However, the relationship of the difference in inhibition % was found related to hydrogen bonds, and this supported that compound **5f** was the most active compound. And the docking studies were in harmony with found works.

6. DISCUSSION

Carcinogenesis has complicated biochemistry, which is hard to treat when it becomes metastatic. Lung cancer is one of the rough type of malignant tumor formation disease, which causes a high rate of mortality. The lung cancer cells are highly invasive and they rapidly metastasize. Subtypes non-small cell lung cancers (NSCLCs) and small cell lung cancers (SCLCs) have an incidence of 80% and 20% percentages. In recent years, target therapies are planned by evaluating the cell characteristics of the subtypes and the physiological status of the disease and the patient. As mechanisms of disease progression in the organism are resolved, the therapy led to blocking pathways and/or inhibiting enzymes/proteins acting in the process [36]. There

Table 2. IC_{50} values of the compounds (µg/mL).

Compounds	A549	NIH/3T3	SI
5a	227.5±3.53	<3.9	1.714
5b	>500	<3.9	-
5c	>500	<3.9	-
5d	>500	<3.9	-
5e	>500	17.00±2	-
5f	<3.9	4.15±0.07	106.410
5g	<3.9	<3.9	100
5h	<3.9	<3.9	100
SD	17.5±0.5	nt	nc

SD: Standard Drug (Cisplatin). **SI:** Selectivity index= 100 x (IC_{50} value of NIH/3T3 / IC_{50} value of A549). **nt**: Not tested, **nc**: not calculated.

Table 3. Annexin V-FITC results* of most active compounds.

-	Q1	Q3	Q2	Q4	Q2+Q4
Control	0.1	99.7	0.1	0.1	0.2
5f	29.6	32.0	34.4	4.0	38.4
5g	26.0	26.9	43.4	3.6	47.0
5h	33.9	19.4	44.2	2.5	46.7
SD	53.6	13.8	27.2	5.4	32.6

Q1: Necrotic cells, **Q2**: Late apoptotic cells, **Q3**: Viable cells, **Q4:** Early apoptotic cells, **Q2+Q4**: Early and Late apoptotic cells. **SD**: Standard Drug (Cisplatin). * A549 cells were incubated with IC_{50} values of compounds and cisplatin for 24 hours. At least 10000 cells were analyzed, and quadrant analysis was performed.

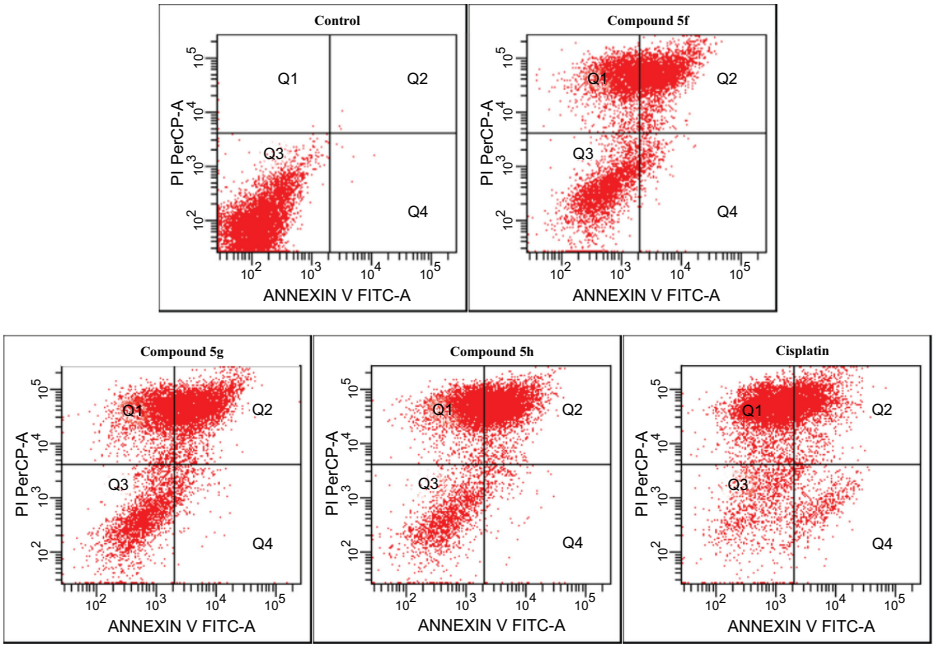


Fig. (1). Apoptosis diagram against A549 cell. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Table 4. Caspase-3 activation.

Groups	P2	P4
Control	99.8	0.2
5f	99.1	0.9
5g	93.5	6.8
5h	97.5	2.4
SD	81.8	18.2

P2: Caspase-3 negative cells%, **P4**: Caspase-3 positive cells%. **SD**: Standard Drug (Cisplatin).

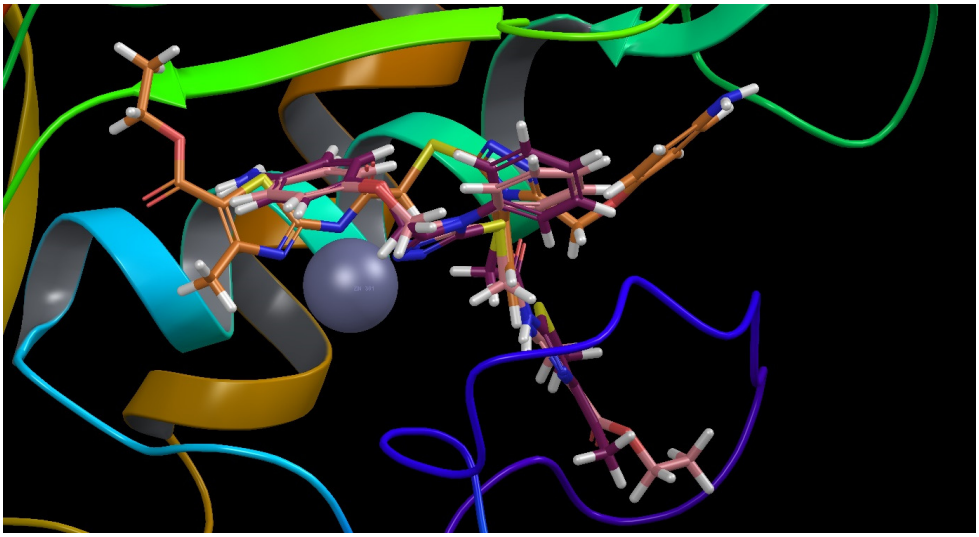


Fig. (2). 3D best docking poses of compounds **5f** (maroon carbons), **5g** (pink carbons) and **5h** (orange carbons) with Zn^{+2} (violet ball) in active site of 5I12. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

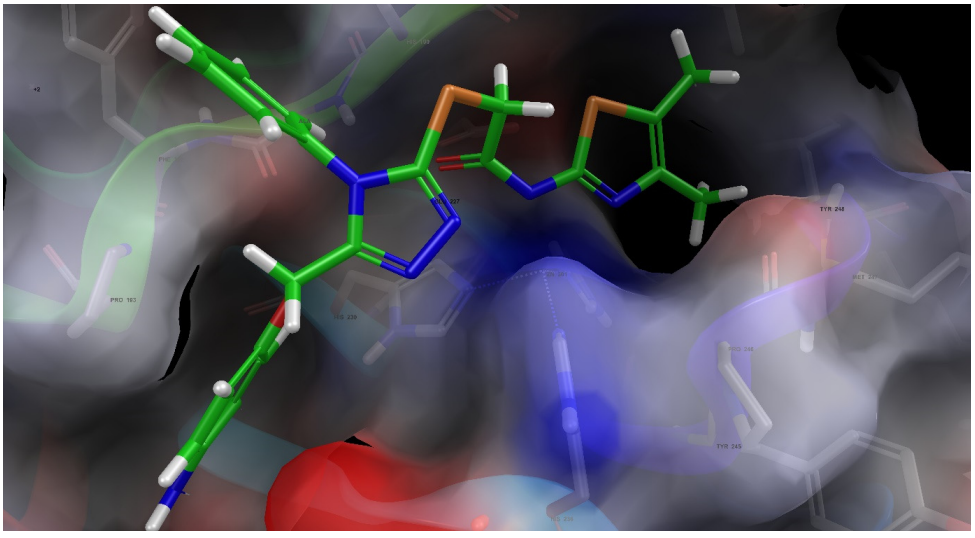


Fig (3). 3D best docking pose of compound **5d** in active site of 5I12. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Table 5. Inhibition% on MMP-9.

-	Inhibition%
5d	11.38 ± 2.52
5f	44.64 ± 2.53
5g	40.18 ± 1.78
5h	37.50 ± 2.53
NNGH	91.10 ± 2.50

100 µg/mL of each synthesized compound was used to determine the inhibition% on MMP-9. **NNGH**: *N*-Isobutyl-*N*-(4-methoxyphenylsulfonyl)glycyl hydroxamic acid, and 1.3 µM/mL of it was used to determine the inhibition% on MMP-9.

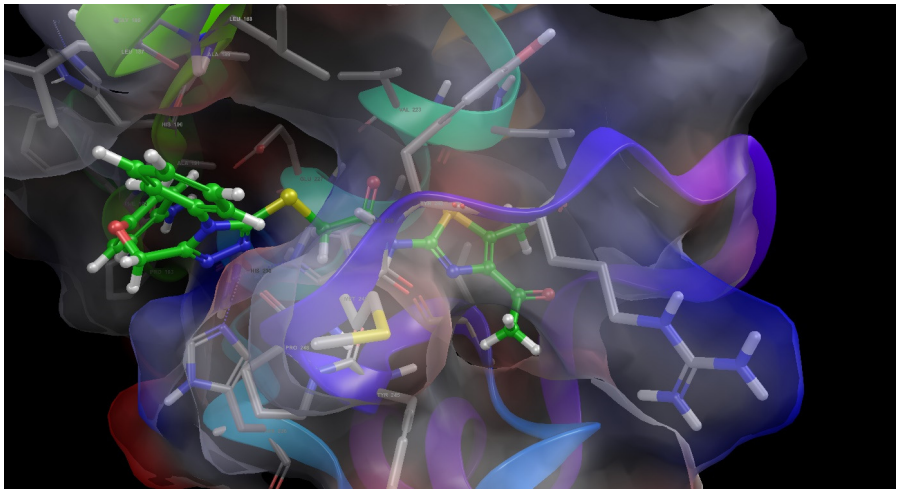


Fig. (4). 3D best docking pose of compound **5f** (green carbons) in active site of 5I12. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

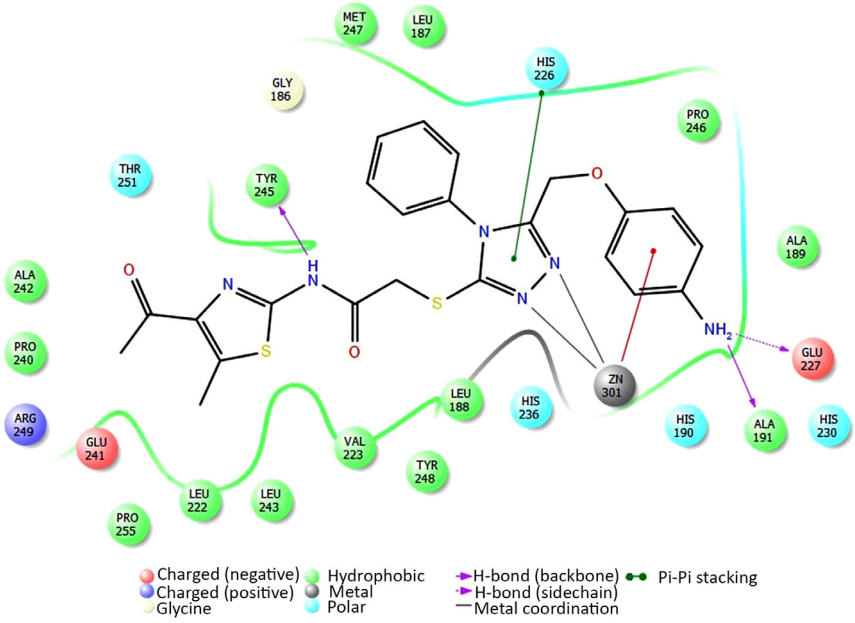


Fig. (5). 2D interaction diagram of compound **5f** in active site of 5I1**,** (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

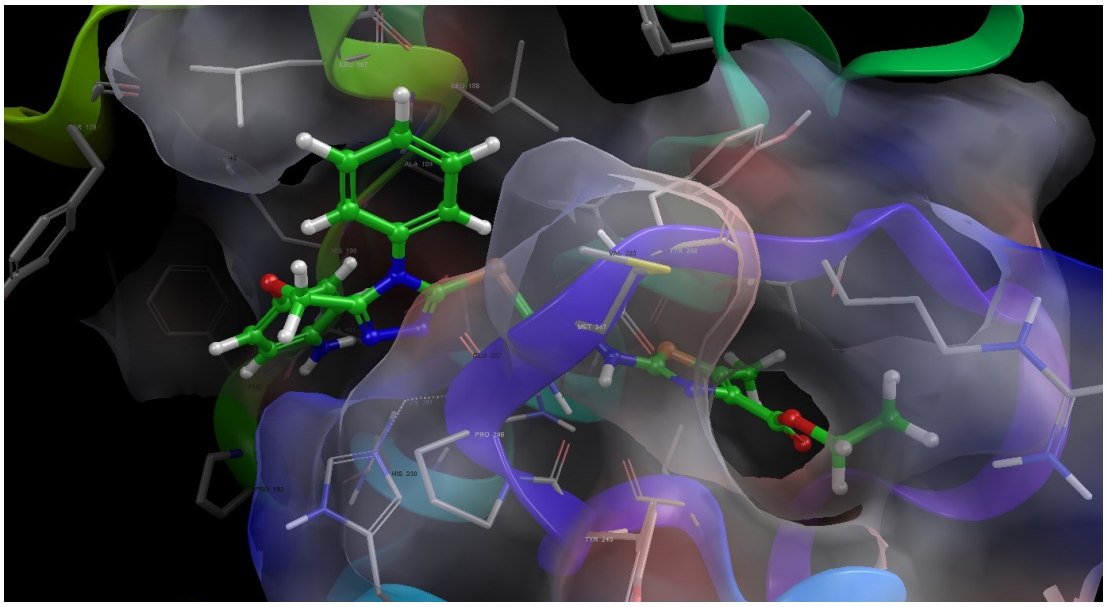


Fig. (6). 3D best docking pose of compound **5g** (green carbons) in active site of 5I12. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

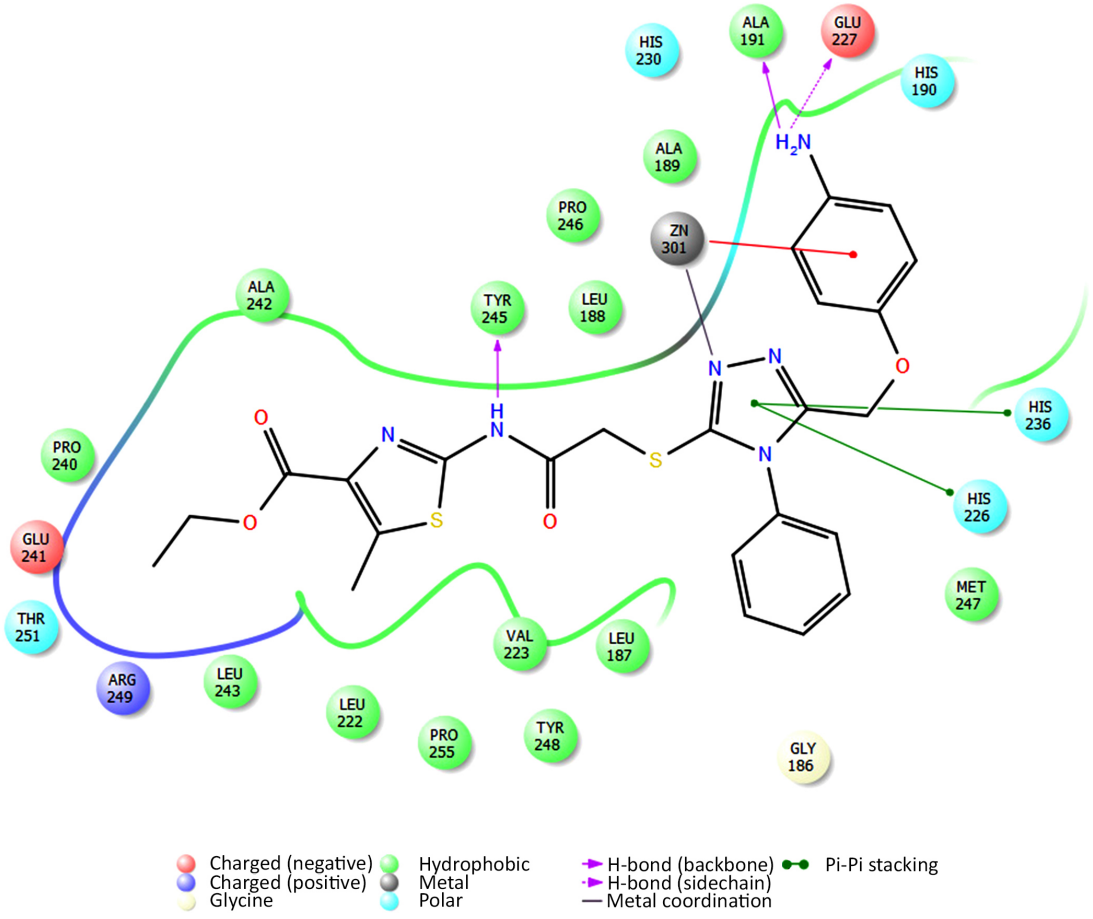


Fig. (7). 2D interaction diagram of compound **5g** in active site of 5I12. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

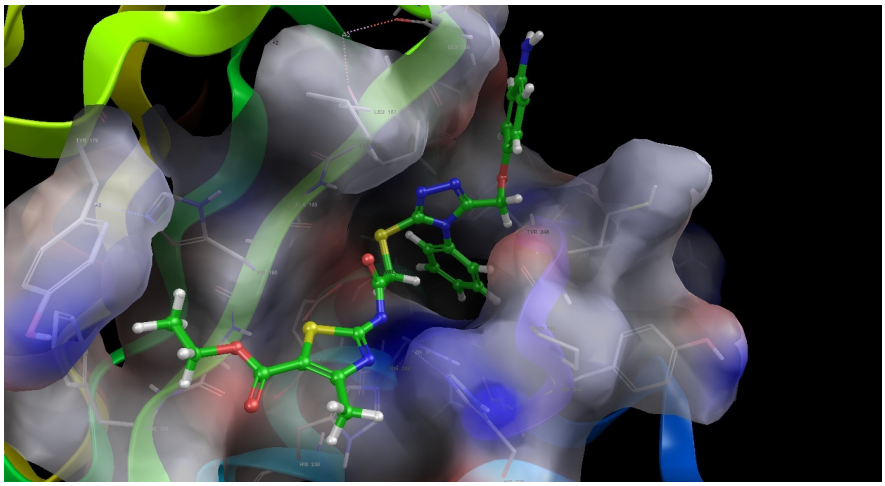


Fig. (8). 3D best docking pose of compound **5h** (green carbons) in active site of 5I12. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

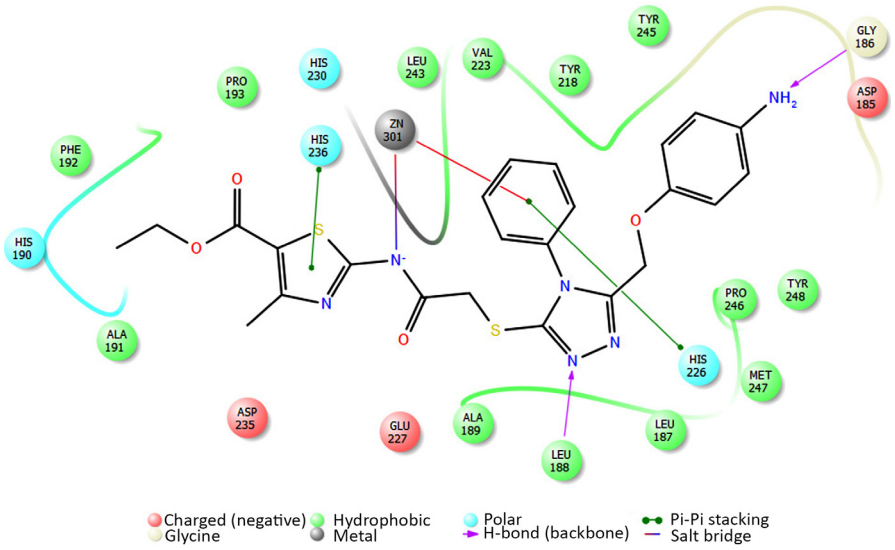


Fig. (9). 2D interaction diagram of compound **5h** in active site of 5I12. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

are different molecular targets in the treatment of NSCLC, which combines molecular targeting agents with cytotoxic agents, based on the underlying molecular mechanisms regulating cell-cycle cells to self-destruct *via* apoptosis. Some of the apoptotic pathways are associated with the activation of executioner caspase(s) 3, 7 and matrix metalloproteinase-9 [37, 38].

When we evaluate the anticancer activity of the compounds **5a-5h**, high cytotoxicity was determined; among them, **5f**, **5g** and **5h** exhibited more significant results, which were comparable to cisplatin. Apoptosis was determined for these three compounds. The final triazole compounds differ from the others due to possessing phenyl and thiazole rings. Thiazole ring, including compounds **5f**, **5g** and **5h** showed high potency. There are many studies based on the anticancer activity of thiazoles, in which our research group worked to determine the mechanism of action considering thiazoles such as ribonucleotide reductase inhibitor [39], aromatase inhibitor [40] and DNA synthesis inhibitor [41].

Considering caspase-3 and MMP-9 inhibition results, compounds did not effect the caspase-3 enzyme. Besides, three cytotoxic compounds **5f**, **5g** and **5h** slightly inhibited the MMP-9 enzyme. To determine the distinction between active and nonactives, compound **5d** was added to the mentioned three compounds and evaluated in the molecular docking program. The compounds **5d**, **5f, 5g** and **5h** were docked to the crystal structure of MMP. In relation to the inhibition percentages on the relative enzyme, compounds **5f**, **5g** and **5h** were interacted amino acids, whereas **5d** did not bind to them (Fig. **3**). When we regard substituents for less or non-active compounds, withdrawing or donating electron groups at phenyl and non-hydrophilic substitutions at thiazole ring (such as 4-phenyl or 4,5-dimethyl) caused a problem due to their bulky and/or hydrophobic affinity. On the other hand, acetyl substituted thiazoles increased anticancer activity *via* MMP-9 inhibition. The reason seems to be its small volume to position for H-bonding. In fact, although ester linkage increased anticancer activity, it decreased MMP-9 inhibition. For this purpose, a substation

on thiazole moiety may diversify with the bioisosteres endowed with H-donor or acceptor substitutions. Additionally, our docking study gave rise to thought right that N substitution on triazole ring may have a short carbon chain with composing π interactions for hydrophobicity and easy placement. In fact, the importance of π-π stacking between the ligand and the imidazole ring of His226 was underlined [35].

Eventually, most of them were found less active or non-effective against lung cancer cells, although final compounds have good pharmacokinetic profiles. But, the discrepancy between the active compounds and others was related to the existence of pentagonal aromatic rings with acyl substituent due to ligand-enzyme active side accordance. This situation supports that thiazole derivatives have determined to have higher efficiency than benzothiazole derivatives. In addition, it was observed that the active molecules were settled in the active site easily, and so facilitated to make the interaction of the rest of the molecule and enzyme. These modifications especially increased anticancer activity through MMP-9 inhibition. Thus, in the next studies, these are untransferable components in designing strategy.

CONCLUSION

Eight new 1,2,4-triazole derivatives bearing 4-aminophenyl moiety were synthesized and fully characterized by spectroscopic analysis. All compounds exhibited high cytotoxicity against tumor cell line, A549 and healthy cell line, NIH/3T3. Only compound **5f** could be evaluated selective to cancer cells. Compounds **5f**, **5g** and **5h** exhibited satisfying apoptosis ratios, which were better than cisplatin provoked. These three compounds were studied to determine the inhibition of enzymes caspase-3 and MMP-9. According to inhibition percentages and molecular docking studies, the mentioned compounds showed antiproliferative activity compatible with virtual results *via* a slight MMP-9 inhibition pathway.

AUTHORS' CONTRIBUTIONS

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The authors confirm that the data supporting the findings of this research are available along with the manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Declared none.

SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's website along with the published article.

REFERENCES

[1] Mernyak, E. Quality of life in chronic disease patients. *Health Psychcol. Res.,* **2013**, *1*(3),

[2] Seyfried, T.N.; Huysentruyt, L.C. On the origin of cancer metastasis. *Crit. Rev. Oncog.,* **2013**, *18*(1-2), 43-73. http://dx.doi.org/10.1615/CritRevOncog.v18.i1-2.40 PMID: 23237552

[3] Wu, P.; Nielsen, T.E.; Clausen, M.H. FDA-approved small-molecule kinase inhibitors. *Trends Pharmacol. Sci.,* **2015**, *36*(7), 422-439. http://dx.doi.org/10.1016/j.tips.2015.04.005 PMID: 25975227

[4] Kumbhare, R.M.; Dadmal, T.L.; Ramaiah, M.J.; Kishore, K.S.; Pushpa Valli, S.N.; Tiwari, S.K.; Appalanaidu, K.; Rao, Y.K.; Bhadra, M.P. Synthesis and anticancer evaluation of novel triazole linked N-(pyrimidin-2-yl)benzo[d]thiazol-2-amine derivatives as inhibitors of cell survival proteins and inducers of apoptosis in MCF-7 breast cancer cells. *Bioorg. Med. Chem. Lett.,* **2015**, *25*(3), 654-658. http://dx.doi.org/10.1016/j.bmcl.2014.11.083 PMID: 25563891

[5] Elmore, S. Apoptosis: A review of programmed cell death. *Toxicol. Pathol.,* **2007**, *35*(4), 495-516. http://dx.doi.org/10.1080/01926230701320337 PMID: 17562483

[6] McIlwain, D.R.; Berger, T.; Mak, T.W. Caspase functions in cell death and disease. *Cold Spring Harb. Perspect. Biol.,* **2013**, *5*(4), a008656. http://dx.doi.org/10.1101/cshperspect.a008656 PMID: 23545416

[7] Ozmen, N.; Kaya-Sezginer, E.; Bakar-Ates, F. The cellular uptake and apoptotic efficiency of colchicine is correlated with downregulation of MMP-9 mRNA expression in SW480 colon cancer cells. *Anticancer. Agents Med. Chem.,* **2018**, *18*(13), 1927-1933. http://dx.doi.org/10.2174/1871520618666180821102047 PMID: 30129419

[8] Mishra, C.B.; Mongre, R.K.; Kumari, S.; Jeong, D.K.; Tiwari, M. Novel triazole-piperazine hybrid molecules induce apoptosis *via* activation of the mitochondrial pathway and exhibit antitumor efficacy in osteosarcoma xenograft nude mice model. *ACS Chem. Biol.,* **2017**, *12*(3), 753-768. http://dx.doi.org/10.1021/acschembio.6b01007 PMID: 28084722

[9] El-Sherief, H.A.M.; Youssif, B.G.M.; Abdelazeem, A.H.; Abdel-Aziz, M.; Abdel-Rahman, H.M. Design, synthesis and antiproliferative evaluation of novel 1,2,4-triazole/schiff base hybrids with EGFR and B-RAF inhibitory activities. *Anticancer. Agents Med. Chem.,* **2019**, *19*(5), 697-706. http://dx.doi.org/10.2174/1871520619666181224115346 PMID: 30582484

[10] Wang, X-F.; Zhang, S.; Li, B-L.; Zhao, J-J.; Liu, Y-M.; Zhang, R-L.; Li, B.; Chen, B-Q. Synthesis and biological evaluation of

disulfides bearing 1,2,4-triazole moiety as antiproliferative agents. *Med. Chem. Res.,* **2017**, *26*(12), 3367-3374.

http://dx.doi.org/10.1007/s00044-017-2029-0

[11] Mioc, M.; Soica, C.; Bercean, V.; Avram, S.; Balan-Porcarasu, M.; Coricovac, D.; Ghiulai, R.; Muntean, D.; Andrica, F.; Dehelean, C.; Spandidos, D.A.; Tsatsakis, A.M.; Kurunczi, L. Design, synthesis and pharmaco-toxicological assessment of 5-mercapto-1,2,4-triazole derivatives with antibacterial and antiproliferative activity. *Int. J. Oncol.,* **2017**, *50*(4), 1175-1183.

http://dx.doi.org/10.3892/ijo.2017.3912 PMID: 28350123

[12] Tokala, R.; Bale, S.; Janrao, I.P.; Vennela, A.; Kumar, N.P.; Senwar, K.R.; Godugu, C.; Shankaraiah, N. Synthesis of 1,2,4-triazole-linked urea/thiourea conjugates as cytotoxic and apoptosis inducing agents. *Bioorg. Med. Chem. Lett.,* **2018**, *28*(10), 1919-1924.

http://dx.doi.org/10.1016/j.bmcl.2018.03.074 PMID: 29657100

[13] Sharma, V.M.; Kumar, G.S.; Reddy, K.V.; Nagalingam, S.; Cunningham, A.M.; Ummanni, K.; Hugel, R.H.; Sharma, D.; Malhotra, V.S. Synthesis and biological evaluation of triazole-vanillin molecular hybrids as anti-cancer agents. *Curr. Bioact.,* **2017**, *13*(3), 223-235.

http://dx.doi.org/10.2174/1573407213666161128122552

[14] Keerthy, H.K.; Mohan, S.; Basappa, ; Bharathkumar, H.; Rangappa, S.; Svensson, F.; Bender, A.; Mohan, C.D.; Rangappa, K.S.; Bhatnagar, R. Basappa; Bharathkumar, H.; Rangappa, S.; Svensson, F.; Bender, A.; Mohan, C.D.; Rangappa, K.S.; Bhatnagar, R. Triazole-pyridine dicarbonitrile targets phosphodiesterase 4 to induce cytotoxicity in lung carcinoma cells. *Chem. Biodivers.,* **2019**, *16*(9), e1900234.

http://dx.doi.org/10.1002/cbdv.201900234 PMID: 31287204

[15] El-Gazzar, Y.I.; Georgey, H.H.; El-Messery, S.M.; Ewida, H.A.; Hassan, G.S.; Raafat, M.M.; Ewida, M.A.; El-Subbagh, H.I. Synthesis, biological evaluation and molecular modeling study of new (1,2,4-triazole or 1,3,4-thiadiazole)-methylthio-derivatives of quinazolin-4(3H)-one as DHFR inhibitors. *Bioorg. Chem.,* **2017**, *72*, 282-292.

http://dx.doi.org/10.1016/j.bioorg.2017.04.019 PMID: 28499189

[16] El-Sherief, H.A.M.; Youssif, B.G.M.; Bukhari, S.N.A.; Abdel-Aziz, M.; Abdel-Rahman, H.M. Novel 1,2,4-triazole derivatives as potential anticancer agents: Design, synthesis, molecular docking and mechanistic studies. *Bioorg. Chem.,* **2018**, *76*, 314-325.

http://dx.doi.org/10.1016/j.bioorg.2017.12.013 PMID: 29227915

[17] Bekircan, O.; Kahveci, B.; Küçük, M. Synthesis and anticancer evaluation of some new unsymmetrical 3, 5-diaryl-4H-1, 2, 4-triazole derivatives. *Turk. J. Chem.,* **2006**, *30*(1), 29-40.

[18] Jia, Y.; Si, L.; Lin, R.; Jin, H.; Jian, W.; Yu, Q.; Yang, S. Thiophenol-formaldehyde triazole causes apoptosis induction in ovary cancer cells and prevents tumor growth formation in mice model. *Eur. J. Med. Chem.,* **2019**, *172*, 62-70.

http://dx.doi.org/10.1016/j.ejmech.2019.03.033 PMID: 30947122

[19] Cihan-Ustundag, G.; Simsek, B.; Ilhan, E.; Capan, G. Synthesis, characterization, antimycobacterial and anticancer evaluation of new 1, 2, 4-triazole derivatives. *Lett. Drug Des. Discov.,* **2014**, *11*(3), 290-296.

http://dx.doi.org/10.2174/157018081131000074

[20] Ahmed, F.F.; Abd El-Hafeez, A.A.; Abbas, S.H.; Abdelhamid, D.; Abdel-Aziz, M. New 1,2,4-triazole-Chalcone hybrids induce caspase-3 dependent apoptosis in A549 human lung adenocarcinoma cells. *Eur. J. Med. Chem.,* **2018**, *151*, 705-722.

http://dx.doi.org/10.1016/j.ejmech.2018.03.073 PMID: 29660690

[21] Bozsity, N.; Minorics, R.; Szabo, J.; Mernyak, E.; Schneider, G.; Wolfling, J.; Wang, H.C.; Wu, C.C.; Ocsovszki, I.; Zupko, I. Mechanism of antiproliferative action of a new d-secoestrone-triazole derivative in cervical cancer cells and its effect on cancer cell motility *J. Steroid. Biochem. Mol. Biol.,* **2017**, *165*(Pt b), 247-257.

[22] Kaur, R.; Dwivedi, A.R.; Kumar, B.; Kumar, V. Recent developments on 1, 2, 4-triazole nucleus in anticancer compounds: A review. *Anticancer. Agents Med. Chem.,* **2016**, *16*(4), 465-489.

http://dx.doi.org/10.2174/1871520615666150819121106 PMID: 26286663

[23] Kulabaş, N.; Tatar, E.; Bingöl Özakpınar, Ö.; Özsavcı, D.; Pannecouque, C.; De Clercq, E.; Küçükgüzel, İ. Synthesis and antiproliferative evaluation of novel 2-(4H-1,2,4-triazole-3-ylthio)acetamide derivatives as inducers of apoptosis in cancer cells. *Eur. J. Med. Chem.,* **2016**, *121*, 58-70.

http://dx.doi.org/10.1016/j.ejmech.2016.05.017 PMID: 27214512

[24] Hugenberg, V.; Riemann, B.; Hermann, S.; Schober, O.; Schäfers, M.; Szardenings, K.; Lebedev, A.; Gangadharmath, U.; Kolb, H.; Walsh, J.; Zhang, W.; Kopka, K.; Wagner, S. Inverse 1,2,3-triazole-1-yl-ethyl substituted hydroxamates as highly potent matrix metalloproteinase inhibitors: (radio)synthesis, *in vitro* and first *in vivo* evaluation. *J. Med. Chem.,* **2013**, *56*(17), 6858-6870.

http://dx.doi.org/10.1021/jm4006753 PMID: 23899323

[25] Ramos-Inza, S.; Aydillo, C.; Sanmartín, C.; Plano, D. Thiazole moiety: An interesting scaffold for developing new antitumoral compounds. *Heterocycles - Synthesis and Biological Activities*.; IntechOpen, **2019**, DOI: 10.5772/intechopen.82741.

DOI: 10.5772/intechopen.82741

[26] Chhabria, M.T.; Patel, S.; Modi, P.; Brahmkshatriya, P.S. Thiazole: A review on chemistry, synthesis and therapeutic importance of its derivatives. *Curr. Top. Med. Chem.,* **2016**, *16*(26), 2841-2862.

http://dx.doi.org/10.2174/1568026616666160506130731 PMID: 27150376

[27] Evren, A.E.; Yurttas, L.; Ekselli, B.; Akalin-Ciftci, G. Synthesis and biological evaluation of 5-methyl-4-phenyl thiazole derivatives as anticancer agents. *Phosphorus Sulfur Silicon Relat. Elem.,* **2019**, *194*(8), 820-828.

http://dx.doi.org/10.1080/10426507.2018.1550642

[28] Lowe, S.W.; Lin, A.W. Apoptosis in cancer. *Carcinogenesis,* **2000**, *21*(3), 485-495.

http://dx.doi.org/10.1093/carcin/21.3.485 PMID: 10688869

[29] Maestro, S. *Version 10.6,* **2016**.

[30] Nuti, E.; Cuffaro, D.; D'Andrea, F.; Rosalia, L.; Tepshi, L.; Fabbi, M.; Carbotti, G.; Ferrini, S.; Santamaria, S.; Camodeca, C.; Ciccone, L.; Orlandini, E.; Nencetti, S.; Stura, E.A.; Dive, V.; Rossello, A. Sugar-based arylsulfonamide carboxylates as selective and water-soluble matrix metalloproteinase-12 inhibitors. *ChemMedChem,* **2016**, *11*(15), 1626-1637.

http://dx.doi.org/10.1002/cmdc.201600235 PMID: 27356908

[31] Daina, A.; Michielin, O.; Zoete, V. SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci. Rep.,* **2017**, *7*, 42717.

http://dx.doi.org/10.1038/srep42717 PMID: 28256516

[32] Daina, A.; Michielin, O.; Zoete, V. iLOGP: a simple, robust, and efficient description of n-octanol/water partition coefficient for drug design using the GB/SA approach. *J. Chem. Inf. Model.,* **2014**, *54*(12), 3284-3301.

http://dx.doi.org/10.1021/ci500467k PMID: 25382374

[33] Daina, A.; Zoete, V. A boiled-egg to predict gastrointestinal absorption and brain penetration of small molecules. *ChemMedChem,* **2016**, *11*(11), 1117-1121.

http://dx.doi.org/10.1002/cmdc.201600182 PMID: 27218427

[34] Lipinski, C.A.; Lombardo, F.; Dominy, B.W.; Feeney, P.J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv. Rev.,* **2001**, *46*(1-3), 3-26.

http://dx.doi.org/10.1016/S0169-409X(00)00129-0 PMID: 11259830

[35] Gao, Q.; Wang, Y.; Hou, J.; Yao, Q.; Zhang, J. Multiple receptor-ligand based pharmacophore modeling and molecular docking to screen the selective inhibitors of matrix metalloproteinase-9 from natural products. *J. Comput. Aided Mol. Des.,* **2017**, *31*(7), 625-641.

http://dx.doi.org/10.1007/s10822-017-0028-3 PMID: 28623487

[36] Yurttaş, L.; Öztürk, Ö.; Cantürk, Z. New procaspase activating compound (PAC-1) like molecules as potent antitumoral agents against lung cancer. *Lett. Drug Des. Discov.,* **2019**, *16*, 645-655.

http://dx.doi.org/10.2174/1570180815666180926113040

[37] Wang, T.H.; Wang, H.S.; Soong, Y.K. Paclitaxel-induced cell death: Where the cell cycle and apoptosis come together. *Cancer,* **2000**, *88*(11), 2619-2628.
http://dx.doi.org/10.1002/1097-0142(20000601)88:11<2619::AID-CNCR26>3.0.CO;2-J PMID: 10861441

[38] Nalla, A.K.; Gorantla, B.; Gondi, C.S.; Lakka, S.S.; Rao, J.S. Targeting MMP-9, uPAR, and cathepsin B inhibits invasion, migration and activates apoptosis in prostate cancer cells. *Cancer Gene Ther.,* **2010**, *17*(9), 599-613.
http://dx.doi.org/10.1038/cgt.2010.16 PMID: 20448670

[39] Ertas, M.; Sahin, Z. BulBul, E.F.; Bender, C.; Biltekin, S.N.; Berk, B.; Yurttas, L.; Nalbur, A.M.; Çelik, H.; Demirayak, Ş. Potent ribonucleotide reductase inhibitors: Thiazole-containing thiosemicarbazone derivatives. *Arch. Pharm. Chem. Life Sci.,* **2019**, *352*, 1-13.

[40] Ertas, M.; Sahin, Z.; Berk, B.; Yurttas, L.; Biltekin, S.N.; Demirayak, Ş. Pyridine-substituted thiazolylphenol derivatives: Synthesis, modeling studies, aromatase inhibition, and antiproliferative activity evaluation. *Arch. Pharm. Chem. Life Sci.,* **2018**, *351(3-4),* e1700272.
[PMID: 29522642]

[41] Yurttaş, L.; Özkay, Y.; Akalın-Çiftçi, G.; Ulusoylar-Yıldırım, Ş. Synthesis and anticancer activity evaluation of N-[4-(2-methylthiazol-4-yl)phenyl]acetamide derivatives containing (benz)azole moiety. *J. Enzyme Inhib. Med. Chem.,* **2014**, *29*(2), 175-184.
http://dx.doi.org/10.3109/14756366.2013.763253 PMID: 23391122