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SYNTHESIS, CHARACTERIZATION, AND CYTOTOXIC PROFILE OF NOVEL 3-(2-((1-phenyl-1H-1,2,3-triazol-4-yl) methylamino) acetyl)-2H-chromen-2-one

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ABSTRACT

Cancer continues to be the most unconquerable and grievous medical condition in developing countries. In the present study, a series of coumarin-triazole hybrid compounds were designed and synthesized for anticancer activity. For all compounds, MTT assay was done by using four different cell lines like Hela (Cervical cancer cell line), MCF-7 (Breast cancer cell line), A-549 (Lung cancer cell line), and IMR-32 (Neuroblastoma cell line). Most of the compounds showed moderate to good efficacy against different cancer cell lines. Among all, the compound **7h** exhibited the best anticancer activity against the MCF-7 cell line.

Keywords: Cancer, Triazole, Coumarin, Cell line, MTT assay

INTRODUCTION

Cancer is one of the most dangerous diseases that cause the death of most of the world due to its uncontrolled growth and division of a cell. Cancer continues to be the most challenging and dangerous disease in developing countries **[1]**. The development of anticancer agents is the main focus of many pharmaceutical and research organizations. Recent growth in molecular science, proteomics, and genomics generated new targets in anticancer drug discovery. Most enzyme binding pockets are susceptible to interact with heterocyclic moieties. Thus, the presence of heterocyclic moiety while designing drug molecules plays a vital role

in interacting with the target and blocking the pathways associated with cancer progression. Heterocycles are widely distributed in nature and play an essential role in the metabolism of living cells. Among all the heterocycles, nitrogen-containing heterocycles are commonly used in case of drug discovery. 84% of small molecular drugs contain at least one nitrogen atom in their core moiety in all drugs. Different N-heterocycles include aziridine, pyrrole, pyrazole, triazole, imidazole, indole, purines, pyrimidines, piperidine.

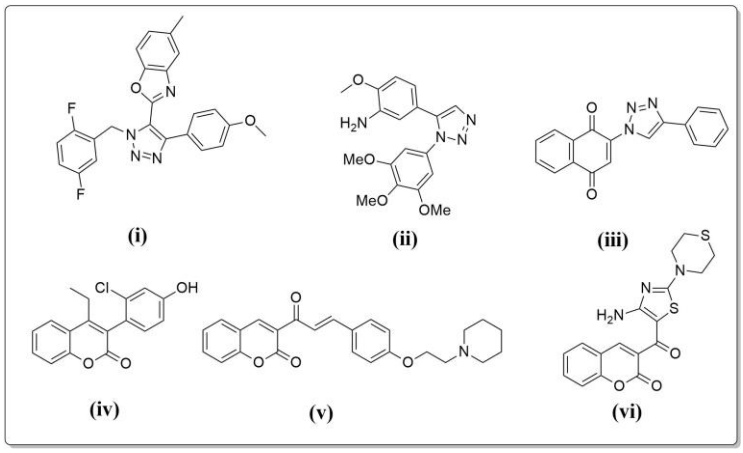
The major problems associated with these anticancer drugs are undesirable effects and drug interactions. For example, in an experimental rat model, Erlotinib significantly reduced the white blood cells (WBC), red blood cells (RBC), and hemoglobin levels. Moreover, increased levels of liver function markers such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), along with damage to the internal organs was also observed **[2]**.

1, 2, 3–triazoles are another important pharmacophore in medicinal chemistry due to their unique drug-like characteristics. They can form conventional hydrogen bonds with biological targets **[3]**. This moiety is stable towards both oxidation and reduction in acidic and basic mediums, which will help to improve solubility and efficacy. Triazole moiety-containing molecules were pharmaceutically important due to their various therapeutic activities like anticancer, **[4, 5]** (**i, ii, &iii in Figure 1**) anti-HIV **[6]**, anti-tubercular **[7]**, and anti-inflammatory **[8]** activities.

The coumarin (benzopyran-2-one, or chromen-2-one) moiety, which shows interesting pharmacological properties, has intrigued chemists and medicinal chemists for decades to explore the natural coumarins or synthetic analogs for their applicability as drugs **[9]**. Coumarin is an oxygen-containing heterocyclic moiety used in drug discovery because of its diverse biological activities and the preparation of dyes **[10]**. Coumarin shows diverse biological activities such as anti-diabetic **[11]**, antioxidant, hepatoprotective **[12]**, anti-inflammatory, anti-bacterial **[13]** antihemorrhagic activity **[14]**, anticoagulant **[15]**, inhibition of lipid peroxidation **[16]**, antimicrobial **[17]**, anti-HIV **[18]**, anti-tubercular **[19]**, anti-Alzheimer's activity **[20]**, DNA gyrase inhibitory activity **[21]**, and anticancer activity **[22, 23]**.

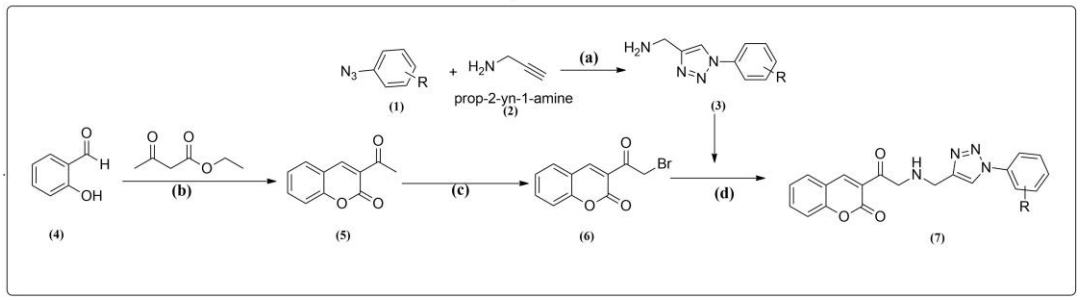
The fight against cancer and the investigation of new drugs to cure the disease has continued in the last few decades. Various researchers tried different therapeutics and therapeutic approaches, but most were withdrawn from the market

due to severe side effects and high toxicity to the normal cells. In discovering new anticancer agents, the molecular hybridization approach is widely used to design small hybrid molecules. The molecular hybridization approach mainly involves a fusion of two or more different pharmaceutically important scaffolds (pharmacophores) in a single molecule **[24]**. These hybrid molecules can have advantages over conventional drugs such as low toxicity **[25]**, high solubility, low Multidrug resistance (MDR), etc. In this study, molecular hybridization approach was used to combine the two bioactive scaffolds (Coumarin and 1, 2, 3-triazole) to get a series of new hybrid compounds. **(Scheme).**



Scheme

Figure 1: Chemical Structures of some reported anticancer agents containing triazole and coumarin moieties



Reaction Conditions: (a) CuI, THF, reflux, 4hr, (b) Pyridine, Ethanol, reflux 2 hr, (c) Br_2, $CHCl_3$, RT, 30 min (d) K_2CO_3, THF, RT, 1 hr.

MATERIALS AND METHODS

Chemistry:

All chemicals and reagents were purchased from Sigma-Aldrich. Column chromatography purifications were performed using silica gel grade 9385, 100−200 mesh, neutral alumina (Sigma). Thin-layer chromatography (TLC) was performed on silica gel 60 F254 plates with 0.20 mm thickness (Merck, Germany), which was visualized under an ultraviolet light chamber (254 and 365 nm). NMR

experiments were run on Bruker Avance III 600 (500 MHz for ^{1}H and 125 MHz for ^{13}C). ^{1}H NMR spectra were recorded for solution in $CDCl_3$ or dimethyl sulfoxide (DMSO) with tetramethylsilane as the standard. Chemical shifts for ^{1}H and ^{13}C spectra were recorded in parts per million (ppm). Data were reported as follows: chemical shift (ppm), integrated intensity, multiplicity (indicated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet), and coupling constants (*J*) in hertz (Hz). MS spectra were obtained on a JEOL MS station 700 (JEOL Ltd., Akishima-Shi, Japan). The FT-IR spectra of the samples were recorded on a JASCO FTIR 4200 spectrometer (JASCO, Easton, MD, USA) using a KBr disk technique. The spectra were recorded from 400 to 4000 cm−1. JASCO software was used for data processing.

General procedure to synthesize compound 3:

To the solution of compound **1** (1 eq) in THF, were added different acetylene **2** (1 eq) and copper iodide (0.2 eq) and heated to reflux temperature (80 ^{0}C) for 4 hrs. After the total consumption of starting material (monitored through TLC), the solvent was evaporated. The reaction mixture was then diluted with ethyl acetate, filtered through a celite bed and washed with water and brine (3×10ml) in a separating funnel. After that, the organic layer was collected and dried over Na_2SO_4 and then evaporated under reduced pressure to get a crude solid which was purified by column chromatography (silica gel 100-200, petroleum ether: ethylacetate 4:1) to afford the desired product as yellow solid with 80-85% yield.

General procedure to synthesize compound 5:

To the solution of compound **4** (1 eq) in ethanol, were pyridine (3 eq) and ethyl 3-oxobutanoate (1 eq) and heated to reflux temperature (80 ^{0}C) for 2 hrs. After the total consumption of starting material (monitored through TLC), the solvent was evaporated. Then, the reaction mixture was diluted with ethyl acetate and washed with water and brine (3×10ml) in a separating funnel. After that, the organic layer was collected and dried over Na2SO4 and then evaporated under reduced pressure to get a crude solid which was purified by column chromatography (silica gel 100-200, petroleum ether: ethylacetate 4:1) to afford the desired product as brown solid with 78-80% yield.

General procedure to synthesize compound 6:

To the solution of compound **5** (1 eq) in chloroform, were added bromine (1 eq) in chloroform added dropwise and continued the reaction at room temperature for 1 hr. After the total consumption of starting material (monitored through TLC), the

solvent was evaporated. Then, the reaction mixture was diluted with ethyl acetate and washed with water and brine (3×10ml) in a separating funnel. After that, the organic layer was collected and dried over Na2SO4 and then evaporated under reduced pressure to get a crude solid which was purified by column chromatography (silica gel 100-200, petroleum ether: ethyl acetate 4:1) to afford the desired product as a white solid with 80-82% yield.

General procedure to synthesize compound 7:

To the solution of compound **6** (1 eq) in THF, were added different compounds **3** (1 eq) and potassium carbonate (1.5 eq) and heated to reflux temperature (80 ^{0}C) for 4 hrs. After the total consumption of starting material (monitored through TLC), the solvent was evaporated. The reaction mixture was then diluted with ethyl acetate, filtered through a celite bed, and washed with water and brine (3×10ml) in a separating funnel. After that, the organic layer was collected and dried over Na2SO4 and then evaporated under reduced pressure to get a crude solid which was purified by column chromatography (silica gel 100-200, petroleum ether: ethyl acetate 4:1) to afford the desired product as yellow solid with 78-92% yield.

Spectral data of the compounds 7a-7j

3-(2-(((1-phenyl-1H-1,2,3-triazol-4-yl)methyl)amino)acetyl)-2H-chromen-2-one (7a)

^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.78 (t, *J* = 0.9 Hz, 1H), 8.50 (s, 1H), 7.76 – 7.63 (m, 4H), 7.55 – 7.46 (m, 3H), 7.40 (ddd, *J* = 8.4, 7.2, 1.6 Hz, 1H), 7.22 – 7.15 (m, 1H), 4.22 (dd, *J* = 5.9, 0.9 Hz, 2H), 4.12 (d, *J* = 6.7 Hz, 2H), 3.68 (tt, *J* = 6.7, 5.9 Hz, 1H).^{13}C NMR (125 MHz) δ 190.30, 161.81, 154.02, 147.07, 144.62, 136.67, 132.84, 130.05, 129.86, 127.59, 125.50 (d, *J* = 4.8 Hz), 122.12, 120.94 (d, *J* = 18.1 Hz), 116.55, 57.67, 40.91. MS: [ESI- m/z] calcd for $C_{20}H_{16}N_4O_3$ $[M+H]^+$ 361.13, found: 365.15; FTIR (KBr) [cm−1] : 3215, 1670,1560,1310, 1270.

3-(2-(((1-(4-fluorophenyl)-1H-1,2,3-triazol-4-yl)methyl)amino)acetyl)-2H-chromen-2-one (7b)

^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.78 (t, *J* = 0.9 Hz, 1H), 8.50 (s, 1H), 7.73 (dd, *J* = 7.7, 1.6 Hz, 1H), 7.67 (td, *J* = 7.5, 1.3 Hz, 1H), 7.62 – 7.55 (m, 2H), 7.49 (dd, *J* = 8.3, 1.2 Hz,1H), 7.40 (ddd, *J* = 8.4, 7.2, 1.6 Hz, 1H), 7.15 – 7.07 (m, 1H), 4.22 (dd, *J* = 5.9, 0.9 Hz, 2H), 4.12 (d, *J* = 6.7 Hz, 2H), 3.68 (tt, *J* = 6.7, 5.9 Hz, 1H).^{13}C NMR (125 MHz) δ 190.30, 163.31, 161.81, 161.34, 154.02, 147.07, 144.62, 133.21 (d, *J* = 2.9 Hz), 132.84, 129.86, 125.50 (d, *J* = 4.8 Hz), 122.95 (d, *J* = 8.3 Hz), 121.85, 121.01, 117.29, 117.11, 116.55, 57.67, 40.91. MS: [ESI- m/z] calcd for $C_{20}H_{16}N_4O_3$ $[M+H]^+$

361.13, found: 365.15; FTIR (KBr) [cm−1] : 3195, 1650,1550,1350, 1240.

3-(2-(((1-(4-chlorophenyl)-1H-1,2,3-triazol-4-yl)methyl)amino)acetyl)-2H-chromen-2-one (7c)

^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.79 (t, *J* = 0.9 Hz, 1H), 8.50 (s, 1H), 7.73 (dd, *J* = 7.7, 1.6 Hz, 1H), 7.67 (td, *J* = 7.5, 1.3 Hz, 1H), 7.53 – 7.46 (m, 3H), 7.40 (ddd, *J* = 8.4, 7.2, 1.6 Hz, 1H), 7.37 – 7.32 (m, 1H), 7.34 (s, 1H), 4.22 (dd, *J* = 5.9, 0.9 Hz, 2H), 4.12 (d, *J* = 6.7 Hz, 2H), 3.68 (tt, *J* = 6.7, 5.9 Hz, 1H).^{13}C NMR (125 MHz) δ 190.30, 161.81, 154.02, 147.07, 144.62, 135.04, 134.75, 132.84, 130.34, 129.86, 125.52, 125.48, 122.24, 121.99, 121.01, 116.55, 57.67, 40.91. MS: [ESI- m/z] calcd for $C_{20}H_{15}ClN_4O_3$ $[M+H]^+$ 395.09, found: 395.05; FTIR (KBr) [cm−1] : 3210, 1690, 1520, 1450, 1420.

3-(2-(((1-(4-bromophenyl)-1H-1,2,3-triazol-4-yl)methyl)amino)acetyl)-2H-chromen-2-one (7d)

^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.78 (d, *J* = 1.0 Hz, 1H), 8.50 (s, 1H), 7.73 (dd, *J* = 7.7, 1.6 Hz, 1H), 7.68 (s, 4H), 7.67 (td, *J* = 7.6, 1.3 Hz, 1H), 7.49 (dd, *J* = 8.3, 1.2 Hz, 1H), 7.43 – 7.37 (m, 1H), 4.22 (dd, *J* = 5.9, 0.9 Hz, 2H), 4.12 (d, *J* = 6.7 Hz, 2H), 3.68 (tt, *J* = 6.7, 5.9 Hz, 1H).^{13}C NMR (125 MHz) δ 190.30, 161.81, 154.02, 147.07, 144.62, 135.20, 133.69, 132.84, 129.86, 125.52, 125.48, 122.08, 121.59, 121.01, 119.71, 116.55, 57.67, 40.91. MS: [ESI-m/z] calcd for $C_{20}H_{15}BrN_4O_3$ $[M+H]^+$ 440.04, found: 440.15; FTIR (KBr) [cm−1] : 3215, 1684, 1530, 1350.

4-(4-(((2-oxo-2-(2-oxo-2H-chromen-3-yl)ethyl)amino)methyl)-1H-1,2,3-triazol-1-yl)benzonitrile (7e)

^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.85 (t, *J* = 0.9 Hz, 1H), 8.50 (s, 1H), 7.95 – 7.89 (m, 2H), 7.89 – 7.84 (m, 2H), 7.73 (dd, *J* = 7.7, 1.6 Hz, 1H), 7.67 (td, *J* = 7.5, 1.3 Hz, 1H), 7.49 (dd, *J* = 8.3, 1.2 Hz, 1H), 7.43 – 7.37 (m, 1H), 4.22 (dd, *J* = 5.9, 0.9 Hz, 2H), 4.12 (d, *J* = 6.7 Hz, 2H), 3.68 (tt, *J* = 6.7, 5.9 Hz, 1H).^{13}C NMR (125 MHz) δ 190.30, 161.81, 154.02, 147.07, 144.62, 137.59, 133.45, 132.84, 129.86, 125.52, 125.48, 122.06, 121.01, 120.82, 118.39, 116.55, 109.18, 57.67, 40.91. MS: [ESI-m/z] calcd for $C_{21}H_{15}N_5O_3$ $[M+H]^+$ 386.12, found: 386.35; FTIR (KBr) [cm−1] : 3220, 2950, 1680, 1570, 1320.

3-(2-(((1-(4-hydroxyphenyl)-1H-1,2,3-triazol-4-yl)methyl)amino)acetyl)-2H-chromen-2-one (7f)

^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.74 (t, *J* = 0.9 Hz, 1H), 8.50 (s, 1H), 8.25 (s, 1H), 7.73 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.67 (td, *J* = 7.5, 1.3 Hz, 1H), 7.57 – 7.51 (m, 2H), 7.49 (dd, *J* = 8.3, 1.2 Hz, 1H), 7.43 – 7.37 (m, 1H), 6.98 – 6.92 (m, 2H), 4.22 (dd, *J* = 5.9, 0.8 Hz, 2H), 4.12 (d, *J* = 6.7 Hz, 2H), 3.68 (tt, *J* = 6.7, 5.9 Hz, 1H).^{13}C NMR (125 MHz) δ 190.30, 161.81, 156.79, 154.02, 147.07, 144.62, 132.84, 129.86,

129.51, 125.52, 125.48, 122.01, 121.79, 121.01, 117.42, 116.55, 57.67, 40.91. MS: [ESI- m/z] calcd for $C_{20}H_{16}N_4O_4$ $[M+H]^+$ 377.12, found: 395.05; FTIR (KBr) [cm−1] : 3225, 1690, 1560, 1490, 1325.

3-(2-(((1-(p-tolyl)-1H-1,2,3-triazol-4-yl)methyl)amino)acetyl)-2H-chromen-2-one (7g):

^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.75 (t, *J* = 0.9 Hz, 1H), 8.50 (s, 1H), 7.73 (dd, *J* = 7.7, 1.6 Hz, 1H), 7.70 – 7.63 (m, 3H), 7.49 (dd, *J* = 8.3, 1.2 Hz, 1H), 7.43 – 7.36 (m, 1H), 7.28 (dd, *J* = 8.8, 0.9 Hz, 2H), 4.22 (dd, *J* = 5.9, 0.9 Hz, 2H), 4.12 (d, *J* = 6.7 Hz, 2H), 3.72 – 3.63 (m, 1H) 2.39 (s,3H).^{13}C NMR (125 MHz) δ 190.30, 161.81, 154.02, 147.07, 144.62, 137.18, 134.64, 132.84, 130.53, 129.86, 125.52, 125.48, 122.11, 121.01, 120.36, 116.55, 57.67, 40.91, 21.07. MS: [ESI- m/z] calcd for $C_{21}H_{18}N_4O_3$ $[M+H]^+$ 375.14, found: 375.15; FTIR (KBr) [cm−1] : 3215, 1685, 1550, 1330, 1070.

3-(2-(((1-(4-methoxyphenyl)-1H-1,2,3-triazol-4-yl)methyl)amino)acetyl)-2H-chromen-2-one (7h)

^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.71 (d, *J* = 0.9 Hz, 1H), 8.50 (s, 1H), 7.73 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.67 (td, *J* = 7.5, 1.3 Hz, 1H), 7.63 – 7.57 (m, 2H), 7.49 (dd, *J* = 8.3, 1.2 Hz, 1H), 7.43 – 7.36 (m, 1H), 7.03 – 6.97 (m, 2H), 4.22 (dd, *J* = 5.9, 0.9 Hz, 2H), 4.12 (d, *J* = 6.7 Hz, 2H), 3.80 (s, 3H), 3.68 (tt, *J* = 6.7, 5.9 Hz, 1H).^{13}C NMR (125 MHz) δ 190.30, 161.81, 159.88, 154.02, 147.07, 144.62, 132.84, 130.80, 129.86, 125.52, 125.48, 122.14, 121.79, 121.01, 116.55, 115.34, 57.67, 55.35, 40.91. MS: [ESI- m/z] calcd for $C_{21}H_{18}ClN_4O_4$ $[M+H]^+$ 391.14, found: 391.15; FTIR (KBr) [cm−1] : 3220, 1680, 1550, 1310, 1190.

3-(2-(((1-(4-(trifluoromethyl)phenyl)-1H-1,2,3-triazol-4-yl)methyl)amino)acetyl)-2H-chromen-2-one (7i):

^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.91 (t, *J* = 0.9 Hz, 1H), 8.50 (s, 1H), 7.91 – 7.84 (m, 2H), 7.80 – 7.71 (m, 3H), 7.67 (td, *J* = 7.5, 1.3 Hz, 1H), 7.49 (dd, *J* = 8.3, 1.2 Hz, 1H), 7.43 – 7.37 (m, 1H), 4.22 (dd, *J* = 5.9, 0.9 Hz, 2H), 4.12 (d, *J* = 6.7 Hz, 2H), 3.68 (tt, *J* = 6.7, 5.9 Hz, 1H).^{13}C NMR (125 MHz) δ 190.30, 161.81, 154.02, 147.07, 144.62, 136.47, 132.84, 127.74, 127.09, 126.83, 125.52, 125.48, 121.97, 121.01, 120.75, 120.72, 120.47, 116.55, 57.67, 40.91. MS: [ESI- m/z] calcd for $C_{21}H_{15}F_3N_4O_3$ $[M+H]^+$ 429.11, found: 429.05; FTIR (KBr) [cm−1] : 3195, 1686, 1560, 1520, 1340.

3-(2-(((1-(4-(trifluoromethoxy)phenyl)-1H-1,2,3-triazol-4-yl)methyl)amino)acetyl)-2H-chromen-2-one (7j):

^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.71 (d, *J* = 0.9 Hz, 1H), 8.50 (s, 1H), 7.73 (dd, *J* = 7.7, 1.6 Hz, 1H), 7.70 – 7.63 (m, 3H), 7.49 (dd, *J* = 8.3, 1.2 Hz, 1H), 7.40 (ddd, *J* = 8.4, 7.2, 1.6 Hz, 1H), 7.30 – 7.24 (m,

2H), 4.22 (dd, *J* = 5.9, 0.9 Hz, 2H), 4.12 (d, *J* = 6.7 Hz, 2H), 3.68 (tt, *J* = 6.8, 5.9 Hz, 1H).^{13}C NMR (125 MHz) δ 190.30, 161.81, 154.02, 147.07, 146.78, 146.74, 146.70, 146.67, 144.62, 132.84, 131.73, 129.86, 125.52, 125.48, 124.08, 123.87, 123.85, 123.83, 123.82, 122.18, 121.99, 121.81, 121.01, 119.89, 117.80, 116.55, 57.67, 40.91. MS: [ESI- m/z] calcd for $C_{21}H_{15}F_3N_4O_4$ $[M+H]^+$ 445.11, found: 445.10; FTIR (KBr) [cm−1] : 3210, 1690, 1555, 1490, 1295, 1190.

Biology

HeLa (cervical), A-549 (lung), MCF-7 (breast), and IMR-32 (neuroblastoma)cell lines were obtained from American Type Culture Collection (ATCC), 10801 University Boulevard Manassas, VA 20110, USA.

MTT Assay:

MTT assay was performed as described. Cells were seeded at a concentration of 1.5× 10^4 cells/ml in a 96 well plate. After overnight incubation, serial concentration of the extracts (12.5µg/ml, 25µg/ml, 50µg/ml & 100µg/ml) were added. Positive control (5-Fluorouracil), Control group was added with 1% ethanol. Each concentration was repeated three times. These cells were incubated in a humidified atmosphere with 5% CO_2 for 3 days. Then 20µl MTT (3-(4, 5- dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) was added to each well and was incubated at 37^0 C for 4 hrs. The medium was removed, formazan was dissolved in DMSO, and the absorbance was taken at 570nm. The growth inhibition was determined using Growth inhibition = (control O.D – sample O.D/ control O.D) and IC_{50} value was determined **[25]**.

Statistical analysis

Results are expressed as mean ± standard deviation (SD).

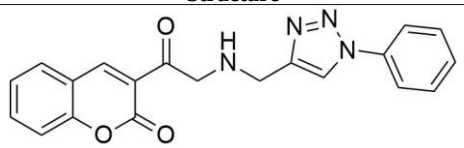
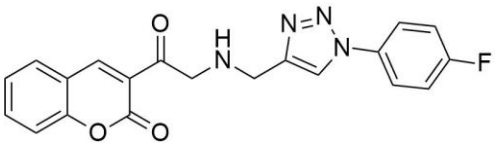
RESULTS AND DISCUSSIONS

The synthesis was started by converting Phenyl azide **1** with different monosubstituted alkynes **2** using the "click" reaction conditions to produce different triazole compounds **3**, in good yields. Coumarin compound **5** was prepared from salicylic acid **4** with ethyl-3-oxobutanoate using pyridine in ethanol, further reacted with bromine to yield compound **6.** Compound **6** coupled with different triazole derivatives **3** in THF at room temperature for 1 hr to give the desired target compounds (**7a-7j**) in 73-94% yield (**Scheme 1**). All the compounds were fully characterized by ^{1}H NMR, ^{13}C NMR, FT-IR, and MS data.

For all the synthesized coumarin-triazole hybrids, cytotoxicity studies were performed. The IC_{50} values of the compounds (**7a-7j**) were determined using the cytotoxicity assay method against four different human cancer cell lines MCF-7 (human breast cancer cell line), HeLa (human cervical cancer cell line, A-549

(human lung cancer cell line), and IMR-32 (human breast cancer cell line). The IC_{50} values were placed in **Table 2** and the marketed drug 5-fluorouracil was used as a positive control. Additional efforts were made to elucidate newly synthesized compounds' structure-activity relationship (SAR) based on cell viability assay results. MTT assay results suggested that most of the compounds were shown moderate to good efficacy against the MCF-7 cell line compared to other cell lines. Among all, compound **7h** exhibited the best activity (IC_{50} value 6.04μM) against the MCF-7 cell line followed by compound **7g** on MCF-7 (IC_{50}=9.54μM) and HeLa (IC_{50}=11.28μM). Compound 7**a** is observed to be potent against MCF-7 (IC_{50} =11.26μM) compared to other cell lines. Compound **7b** is potent against A 549 with an IC_{50} value of 20.17 μM and compound **7f** showed potent anticancer activity against IMR-32 cancer cell lines with IC_{50}values of 13.85μM. Moreover, from the cell viability test results compounds with electron-donating groups at the para position to the triazole attached phenyl ring and unsubstituted phenyl ring derivatives (**7a, 7f, 7g, and 7h**) showed better activity compared to other substitutions, i.e., the electron-withdrawing group at para-position to the triazole attached phenyl ring. The compound 7h showed better activity when compared to other derivatives against four different cancer cell lines. This activity may be due to the electron-donating $–OCH_3$ group at the para position of triazole attached phenyl ring. That compound may form strong Hydrogen bond interaction with the receptor/enzyme active site, which is responsible for better anticancer activity. The above results strongly suggested that electron-donating groups at the para position to the triazole attached phenyl ring and unsubstituted phenyl ring are necessary for the anticancer activity.

Table 1: Structures and physical data of the synthesized compounds *7a-7j*

S. No.	IUPAC name	Structure	Yield
7a	3-(2-(((1-phenyl-1H-1,2,3-triazol-4-yl)methyl)amino)acetyl)-2H-chromen-2-one		87%
7b	3-(2-(((1-(4-fluorophenyl)-1H-1,2,3-triazol-4-yl)methyl)amino)acetyl)-2H-chromen-2-one		89%

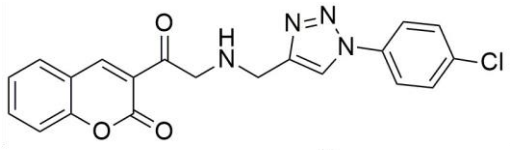
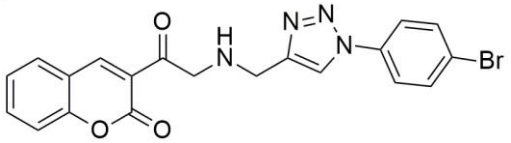
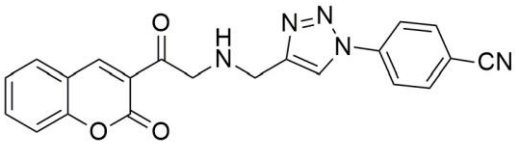
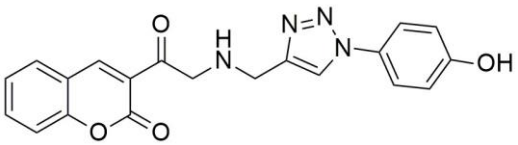
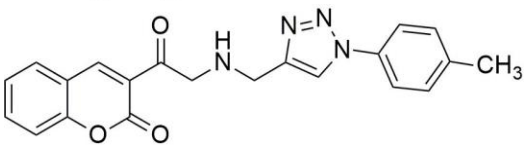
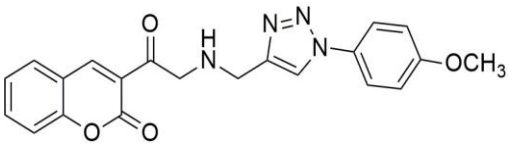
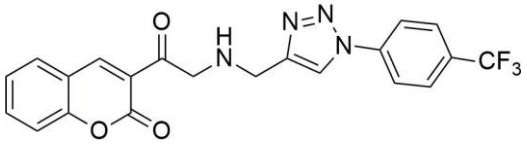
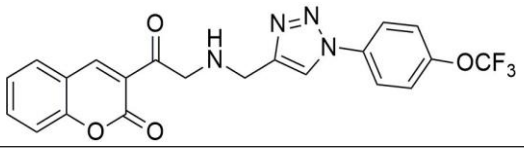
7c	**3-(2-(((1-(4-chlorophenyl)-1H-1,2,3-triazol-4-yl)methyl)amino)acetyl)-2H-chromen-2-one**		**86%**
7d	**3-(2-(((1-(4-bromophenyl)-1H-1,2,3-triazol-4-yl)methyl)amino)acetyl)-2H-chromen-2-one**		**90%**
7e	**4-(4-(((2-oxo-2-(2-oxo-2H-chromen-3-yl)ethyl)amino)methyl)-1H-1,2,3-triazol-1-yl)benzonitrile**		**84%**
7f	**3-(2-(((1-(4-hydroxyphenyl)-1H-1,2,3-triazol-4-yl)methyl)amino)acetyl)-2H-chromen-2-one**		**89%**
7g	**3-(2-(((1-(p-tolyl)-1H-1,2,3-triazol-4-yl)methyl)amino)acetyl)-2H-chromen-2-one**		**92%**
7h	**3-(2-(((1-(4-methoxyphenyl)-1H-1,2,3-triazol-4-yl)methyl)amino)acetyl)-2H-chromen-2-one**		**91%**
7i	**3-(2-(((1-(4-(trifluoromethyl)phenyl)-1H-1,2,3-triazol-4-yl)methyl)amino)acetyl)-2H-chromen-2-one**		**87%**
7j	**3-(2-(((1-(4-(trifluoromethoxy)phenyl)-1H-1,2,3-triazol-4-yl)methyl)amino)acetyl)-2H-chromen-2-one**		**84%**

Table 2: Cytotoxicity Activities of Compounds 7a−7j against Hela, MCF-7, A-549, and IMR-32cell lines in Micromolar

Substituent group (R)	Compound	A 549	HeLa	IMR-32	MCF-7
H	**7a**	**23.71±2.25**	**19.12±1.28**	**40.12±2.08**	**11.26±2.17**
F	**7b**	**20.17±2.11**	**23.55±1.37**	**43.24±3.12**	**27.1±4.19**
Cl	**7c**	**>100**	**>100**	**47.12±1.21**	**54.23±2.18**
Br	**7d**	**>100**	**>100**	**>100**	**>100**
CN	**7e**	**>100**	**>100**	**>100**	**>100**
OH	**7f**	**23.25±1.13**	**16.07±2.13**	**13.85±1.16**	**17.08±1.33**
CH_3	**7g**	**17.39±2.33**	**11.28±0.87**	**19.14±0.88**	**9.54±1.27**
OCH_3	**7h**	**19.11±1.12**	**10.1±1.25**	**16.40±2.17**	**6.042±2.12**
CF_3	**7i**	**>100**	**>100**	**>100**	**>100**
OCF_3	**7j**	**>100**	**>100**	**>100**	**>100**

CONCLUSION

A small set of ten Coumarin-triazole hybrid molecules were synthesized as anticancer agents. The preliminary assay (cell viability assay) results suggested that most of the designed compounds had moderate to good antiproliferative effects against four different cell lines namely, Hela (Cervical cancer cell line), MCF-7 (Breast cancer cell line), A-549 (Lung cancer cell line) and IMR-32 (Neuroblastoma cell line). Compound **7h** showed the best antiproliferative effect against the MCF-7 cell line compared to other molecules in the series. From all the above evidence, it may be concluded that compound **7h** acts as possibly a promising drug candidate for cancer treatment.

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