Research Article

Microwave-assisted one pot synthesis of fused [1,2,3]triazolo-pyrano[3,2-*h*]quinolines and their biological evaluation

Ramesh Babu H.[a], M. Ravinder[b], Sirassu Narsimha[b]*

[a]*Department of Physical Sciences/Chemistry, Kakatiya Institute of Technology and Science, Warangal, T. S-506015, India*

[b]*Department of Chemistry, Chaitanya Degree College (Autonomous), Warangal, TS -506 001, India*

Received: 28 July 2019 Revised: 22 August 2019 Accepted: 27 August 2019

Abstract

Objectives: Microwave-assisted one-pot synthesis of fused [1,2,3]triazolyl[4',5':4,5]pyrano[3,2-h]quinolines and evaluation of their anticancer activity. **Materials and Methods**: The newly synthesized compoundswere characterized by spectroscopic (FTIR, ^{1}H NMR, ^{13}C NMR and Mass) analysis after synthesis. All compounds were screened for their*in vitro* cytotoxic activity against MCF-7, A-549, and HeLa tumor cell lines using MTT analysis. **Results:** The results of cytotoxic activity revealedthat compound **3j**has shown potent activity against MCF-7 and HeLa with IC_{50} values 16.88 ± 0.5 & 11.42 ± 0.6 μM, which are comparable to the standard drug, doxorubicin. **Conclusions:** We have developed a new method for the synthesis of fused 1,2 3-triazole derivatives and the protocol involves a Cu-catalyzed one pot [3+2] cycloaddition followed by C-C bond coupling reaction under microwave condition. Most of the analogues showed strong activity against HeLa and moderate activity against MCF-7 and A-549. The results indicate that these compounds have the potential to develop as leads, and their additional simple structural modifications in the title compounds can produce promising anti-cancer agents for the human cervical cancer HeLa cell line.

***Keywords**:* Microwave synthesis, fused [1,2,3]triazole, anticancer activity

Introduction

1,2,3-Triazoles are an important class of heterocycles that exhibit a wide range of structural and biological activities and are widely used in organic, medicinal, and materials science (Fournier et al., 2007;Hartmuth and Sharpless, 2003;Michael et al., 2008; Alvarez et al., 1994; Tienanet al., 2004; Angell and Burgess, 2007). As compounds containing fused 1,2,3-triazoles become increasingly common in anticancer active substances (Sheng-Jiao et al., 2010; Chung-Yu et al., 2013; Kishna et al., 2015; Narsimha et al., 2016a, 2018b) (**Figure 1**). Based on their structural and biological importance of fused 1,2,3-triazoles, new strategies to synthesize this class of molecules are highly desirable. There are numerous methods available for the synthesis of 1, 4, 5-trisubstituted 1,2,3-triazoles. A classical method involves C-C bond formation of 1, 4-disubstituted 1,2,3-triazole with aryl halide using palladium or copper catalysts in high temperature and longtime refluxing condition (Ackermann et al., 2008, 2009, 2010; Panteleev et al., 2010; Rajkumar et al., 2012).

On the other hand, the quinoline nucleus is an important scaffold found in a variety of biologically active compounds, including natural products and synthetic drugs (Musiol et al., 2007, 2010; Michael, 2007; Kaur et al., 2010). Quinoline and its derivatives have been described as antibacterial (Desai et al., 2012), anticancer (Atwell et al., 1989) and antimalarial (Insuasty et al., 2013). The association of quinoline with the 1,2,3-triazole ring has recently led to the synthesis of new antimicrobial and antitubercular drugs (Sumangala et al., 2010; Thomas et al., 2010, 2011; Harjinder et al., 2014)(**Figure 2**).

Based on the above successful synthesis of fused 1,2,3-triazole derivatives via intramolecular C–H arylation of *in situ* generated 1,4-disubstituted 1,2,3-triazoles and previous therapeutic properties of triazoles and quinolines, as well as continuation of our research on 1,2,3-triazoles (Narsimha et al., 2014, 2016b, 2018a; Ranjithet al., 2017; Swamy et al., 2016, 2017, 2017a; Vasudeva Reedy et al., 2016a, 2016b), we report an efficient method for the synthesis of fused hetrocyclic derivatives having triazole and quinoline

***Address for Corresponding Author:**
Dr. Sirassu Narsimha,
Department of Chemistry, Chaitanya Degree College (Autonomous), Warangal, TS -506 001, India
E-mail: narsimha.s88@gmail.com

DOI: https://doi.org/10.31024/ajpp.2019.5.6.17

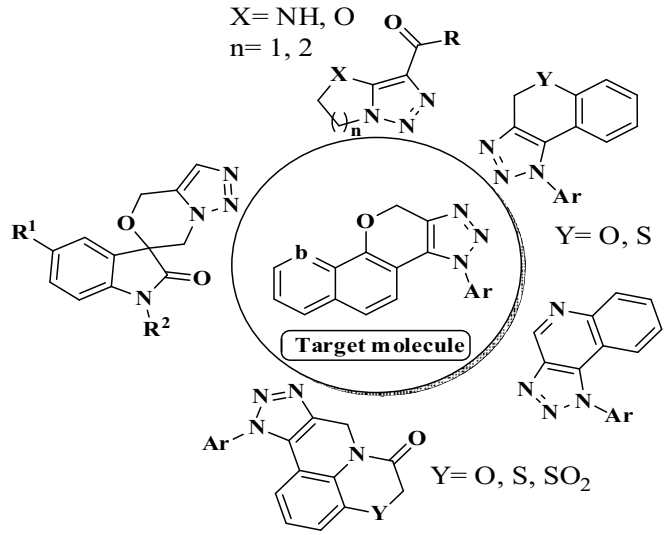


Figure 1. Representative examples of anticancer active fused 1,2,3-triazole moieties

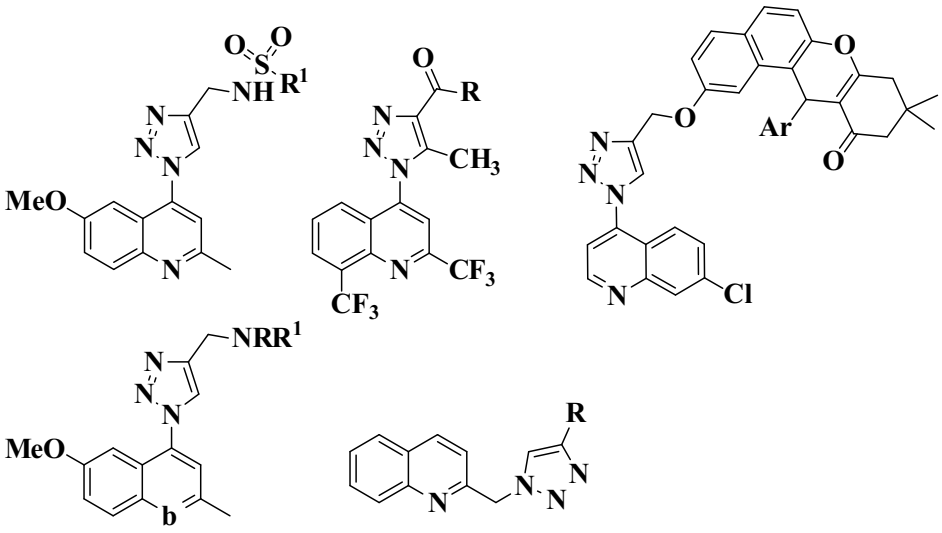
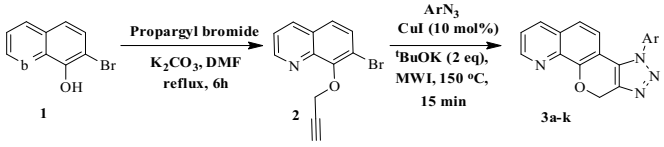


Figure 2. Representative examples of bioactive quinolinecontaining 1,2,3-triazole moieties

moieties in a single scaffold by using copper and palladium catalyzed intramolecular C–H arylation of *in situ* generated 1,4-disubstituted 1,2,3-triazoles from 7-bromo-8-(prop-2-ynyloxy) quinoline with different aryl azides in one pot microwave irradiation method and evaluated for their anticancer activity (**Scheme 1).**



Scheme 1. Synthesis of novel fused [1,2,3]triazolyl[4',5':4,5] pyrano[3,2-h]quinolines

Materials and methods

All the reagents and solvents were purchased from Aldrich/Merck and used without further purifications. Thin-layer chromatography (TLC) was performed using Merck silica gel 60 F254 precoated plates (0.25 mm) and Silica gel (100-200 mesh) was used for column chromatography. The progress of the reactions as well as purity of the compounds was monitored by thin layer chromatography with using ethylacetate /hexane (7/3) as eluent. Melting points were determined using a Cintex apparatus and are uncorrected. IR spectra (KBr pellet) were recorded on a Perkin-Elmer BX seriesFT-IR spectrometer. 400 MHz and 100 MHz NMR spectrometers were used to get ^{1}H-NMR and ^{13}C NMR spectra respectively. Coupling constant (*J*) values are presented in Hertz, spin multiples are given as s (singlet), d (doublet), t (triplet), and m (multiplet). Mass spectra were recorded by using ESI-MS method.

Synthesis and Spectral data

Synthesis of 7-bromo-8-(prop-2-ynyloxy) quinoline (2)

To a mixture of 7-bromoquinolin-8-ol (0.02 mol) andK_2CO_3 (0.05 mol) in DMF (50 ml) was added propargylbromide (0.025 mol) and stirred at room temperature for 6h.After completion of the reaction by TLC analysis, the reaction mixture was poured carefully into ice-cold water (25 mL)and the product was extracted with ethyl acetate (2 x 50 mL). The combined organic layer was dried over anhydrous Na_2SO_4. After filtration, the solvent was evaporated under vacuum and the crude product obtained was purified by column chromatography to afford the pure desired product**2**(86%).Yellow solid.^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.72 (d, *J* = 8.6 Hz, 1H), 8.32 (d, *J*=8.0 Hz, 1H), 7.84 (d, *J*=8.4 Hz, 1H), 7.69-7.74 (m, 1H), 7.42 (d, *J* =8.4Hz, 1H), 4.89 (d, *J* =2.2Hz, O-CH_2, 2H), 2.77 (t, *J*= 2.5 Hz, 1H, CH); ESI-MS: 262 [M+2H].

Synthesis of 3-aryl-3,11-dihydro-[1,2,3]triazolo[4',5':4,5] pyrano [3,2-h]quinoline(3a-k)

7-bromo-8-(prop-2-ynyloxy) quinoline (1mmol), aryl azide (1.2mmol) and ^{t}BuOK (2mmol) were suspended inDMF (10 mL) in a 25-mL glass vial equipped with a small magnetic stirring bar. To this was added copperiodide (10mol %), and the vial was tightly sealed with an aluminum/Teflon crimp top. The mixture was heated at 150°C for 30 min. Progress of the reaction was monitored by TLC. Then, the vial was cooled to room temperature and the reaction mixture was poured carefully into ice-cold water (10 mL) and the product was extracted with ethyl acetate (2 x 15 mL). The combined organic layer was dried over anhydrous Na_2SO_4. After filtration, the solvent was evaporated under vacuum and the crude product obtained was purified by column chromatography (hexane/ethyl acetate gradient) to afford the pure desired product.

3-phenyl-3,11-dihydro-[1,2,3]triazolo[4',5':4,5]pyrano[3,2-h]quinoline (3a):pale red solid; IR(KBr, cm^{-1})ν_{max}3074 (Ar-H),1638 (C=N),1567(C=C); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H8.89 (d,*J*=8.6 Hz,1H), 8.35 (d, *J*=8.0 Hz, 1H),7.72- 7.83 (m,3H), 7.60-7.67 (m, 2H), 7.47-7.53 (m, 2H), 7.27-7.36(m, 1H), 5.62 (s, 2H, O-C***H*****$_2$**); ^{13}C-NMR(100 MHz, $CDCl_3$): δ_C154.3, 153.2, 144.4, 139.3, 133.4, 131.1, 130.3, 128.9,124.6, 122.5,121.6,120.4,118.7,61.7; ESI-MS: 301[M+H]$^+$;Anal. Calcd for $C_{18}H_{12}N_4O$: C, 71.99; H, 4.03; N, 18.66. Found: C, 71.86; H, 4.11; N, 18.79.

3-(4-methoxyphenyl)-3,11-dihydro-[1,2,3]triazolo[4',5':4,5]pyrano[3,2-h]quinoline(3b): White solid; IR(KBr, cm^{-1})ν_{max}3074 (Ar-H), 1638 (C=N),1567(C=C);^{1}H NMR (400 MHz, $CDCl_3$): δ_H 8.75 (d, *J*=8.2 Hz, 1H), 8.32 (d, *J*=8.0 Hz, 1H),7.69-7.78(m, 1H), 7.68 (d, *J*= 8.6 HZ, 2H), 7.46-7.53(m, 1H), 7.27-7.31 (m, 1H), 7.02 (d, *J*= 8.6 HZ, 2H), 5.65 (s, 2H, O-C***H*****$_2$**), 3.86 (s, -OCH_3, 3H); ^{13}C-NMR(100 MHz, $CDCl_3$): δ_C 158.1, 153.7, 144.1, 138.9, 133.5, 132.2, 131.0, 127.8, 124.1, 121.9, 121.2, 119.8, 118.2, 61.5, 57.6; ESI-MS: 331 [M+H]$^+$;Anal. Calcd for $C_{19}H_{14}N_4O_2$: C, 69.08; H, 4.27; N, 16.96. Found: C, 68.09; H, 4.19; N, 17.04.

3-(3-methoxyphenyl)-3,11-dihydro-[1,2,3]triazolo[4',5':4,5]pyrano[3,2-h]quinoline(3c): White solid; IR(KBr, cm^{-1})ν_{max}3074 (Ar-H), 1638 (C=N),1567(C=C); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 8.73 (d, *J*=8.2 Hz, 1H), 8.31 (d, *J*=7.8 Hz, 1H),7.76-7.84 (m, 2H), 7.56-7.62 (m, 2H), 7.34-7.42 (m, 2H), 7.20 (s, 1H), 5.62 (s, 2H, O-C***H*****$_2$**), 3.88 (s, -OCH_3, 3H); ^{13}C-NMR(100 MHz, $CDCl_3$): δ_C 157.1, 153.8, 144.6, 138.2, 134.1, 133.0, 131.4, 128.1, 125.3, 124.6, 123.4, 122.7, 121.3, 120.1, 119.4, 117.6, 61.3, 57.3; ESI-MS: 331 [M+H]$^+$;Anal. Calcd for $C_{19}H_{14}N_4O_2$: C, 69.08; H, 4.27; N, 16.96. Found: C, 69.00; H, 4.21; N, 17.01.

3-(4-chlorophenyl)-3,11-dihydro-[1,2,3]triazolo[4',5':4,5] pyrano[3,2-h]quinoline(3d):

Pale yellow solid; IR(KBr, cm^{-1})ν_{max}3069 (Ar-H), 1641 (C=N),1558(C=C),1329 (C-Cl); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 8.86 (d, *J*=8.6 Hz, 1H), 8.30 (d, *J*=8.2 Hz, 1H), 7.75-7.88(m, 3H), 7.56-7.64(m, 2H), 7.33-7.43 (m, 2H), 5.65 (s, 2H, O-C***H*****$_2$**); ^{13}C-NMR(100 MHz, $CDCl_3$): δ_C 153.2, 144.4, 137.8, 136.2, 132.4, 131.6, 127.5, 126.0, 123.3, 122.3, 120.9, 120.1, 119.8, 118.6, 116.4, 62.0; ESI-MS: 335[M+H]$^+$;Anal. Calcd for $C_{18}H_{11}ClN_4O$: C, 64.58; H, 3.31; N, 16.74. Found: C, 64.52; H, 3.25; N, 16.87.

3-(3-chlorophenyl)-3,11-dihydro-[1,2,3]triazolo[4',5':4,5] pyrano[3,2-h]quinoline*(3e):*

Pale yellow solid; IR(KBr, cm^{-1})ν_{max}3084 (Ar-H), 1668 (C=N),1549(C=C),1341 (C-Cl); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 8.82 (d, *J*=8.0 Hz, 1H), 8.33 (d, *J*=7.6 Hz, 1H), 7.88-7.94(m, 2H), 7.52-7.63(m, 2H), 7.30-7.42 (m, 3H), 5.62 (s, 2H, O-C***H*****$_2$**); ^{13}C-NMR(100 MHz, $CDCl_3$): δ_C 153.6, 144.6, 138.1, 137.0, 133.6, 132.8, 127.6, 126.4, 124.8, 124.2, 123.6, 122.9, 121.4, 120.4, 119.8, 117.3, 116.9, 61.8; ESI-MS: 335 [M+H]$^+$; Anal. Calcd for $C_{18}H_{11}ClN_4O$: C, 64.58; H, 3.31; N, 16.74. Found: C, 64.49; H, 3.22; N, 16.85.

3-(4-nitrophenyl)-3,11-dihydro-[1,2,3]triazolo[4',5':4,5] pyrano[3,2-h]quinoline(3f):

Yellow solid; IR (KBr, cm^{-1})ν_{max}, 3064 (Ar-H), 1653 (C=N),1555 (C=C),1351 (NO2); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 8.89 (d, *J*=8.0 Hz, 1H), 8.32 (d, *J*=7.2 Hz, 1H), 8.22 (d, *J*=2.4 Hz, 1H),8.10 (d, *J* =8.2Hz, 1H), 7.71-7.83(m, 2H), 7.54-7.69(m, 2H), 7.34-7.46 (m, 1H), 5.68 (s,

2H, O-C***H***$_2$); ^{13}C-NMR(100 MHz, $CDCl_3$): δ_C 153.7, 148.1, 144.7, 138.9, 134.6, 132.7, 131.8, 129.6, 127.8, 125.4, 124.2, 123.8, 121.3, 120.4, 116.7, 62.2; ESI-MS: 346[M+H]$^+$; Anal. Calcd for $C_{18}H_{11}N_5O_3$: C, 62.61; H, 3.21; N, 20.28. Found: C, 62.54; H, 3.14; N, 20.37.

3-(3-nitrophenyl)-3,11-dihydro-[1,2,3]triazolo[4',5':4,5] pyrano[3,2-h]quinoline(3g)

White solid; IR (KBr, cm^{-1})ν_{max}3074 (Ar-H), 1639 (C=N),1565(C=C), 1340 (C-NO_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 8.83 (d, *J*=8.6 Hz, 1H), 8.35 (d, *J*=8.0 Hz, 1H), 8.21 (s, 1H),8.07 (m, 1H), 7.76-7.88 (m, 1H), 7.58-7.60 (m, 2H), 7.29-7.56 (m, 2H), 5.69 (s, 2H, O-C***H***$_2$); ^{13}C-NMR(100 MHz, $CDCl_3$): δ_C 153.4, 148.3, 144.7, 138.3, 135.0, 132.4, 131.3, 129.4, 127.6, 125.8, 124.7, 124.0, 123.8, 122.2, 121.6, 120.8, 119.2, 62.5; ESI-MS: 346 [M+H]$^+$; Anal. Calcd for $C_{18}H_{11}N_5O_3$: C, 62.61; H, 3.21; N, 20.28. Found: C, 62.53; H, 3.11; N, 20.35.

3-(p-tolyl)-3,11-dihydro-[1,2,3]triazolo[4',5':4,5] pyrano[3,2-h]quinoline (3h)

Pale yellow solid; IR(KBr, cm^{-1})ν_{max}3062 (Ar-H), 1641 (C=N),1557(C=C); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 8.80 (d, *J*=8.0 Hz, 1H), 8.27 (d, *J*=7.2 Hz, 1H), 7.72-7.88 (m, 2H), 7.52-7.62 (m, 2H),7.20-7.50 (m, 3H), 5.62 (s, 2H, O-C***H***$_2$), 2.39 (s, 3H, -CH_3); ^{13}C-NMR(100 MHz, $CDCl_3$): δ_C 153.1, 143.5, 138.4, 133.4, 131.3, 127.8, 124.3, 123.4, 122.7, 122.1, 120.8, 120.1, 119.6, 118.7, 61.5, 21.3; ESI-MS: 315[M+H]$^+$; Anal. Calcd for $C_{19}H_{14}N_4O$: C, 72.60; H, 4.49; N, 17.82. Found: C, 72.66; H, 4.42; N, 17.77.

3-(m-tolyl)-3,11-dihydro-[1,2,3]triazolo[4',5':4,5] pyrano[3,2-h]quinoline (3i)

Pale yellow solid; IR (KBr, cm^{-1})ν_{max}3080 (Ar-H), 1639 (C=N),1577(C=C); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 8.82 (d, *J*=8.0 Hz, 1H), 8.26(d, *J*=7.8 Hz, 1H), 7.65-7.76 (m, 2H), 7.54-7.62 (m, 2H),7.42-7.48 (m, 1H), 7.30-7.37 (m, 2H), 5.65 (s, 2H, O-C***H***$_2$), 2.43 (s, 3H, -CH_3); ^{13}C-NMR(100 MHz, $CDCl_3$): δ_C 153.5,143.7, 138.7, 133.6, 132.1, 128.1, 124.7, 123.3, 122.8, 120.6, 120.0, 119.4, 118.6, 61.7, 19.8; ESI-MS: 315 [M+H]$^+$; Anal. Calcd for $C_{19}H_{14}N_4O$: C, 72.60; H, 4.49; N, 17.82. Found: C, 72.69; H, 4.45; N, 17.73.

3-(3,5-dimethylphenyl)-3,11-dihydro-[1,2,3]triazolo[4',5':4,5] pyrano[3,2-h]quinoline(3j) Pale yellow solid; IR (KBr, cm^{-1})ν_{max}3066 (Ar-H), 1644 (C=N),1564(C=C); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H8.83 (d, *J*=8.6 Hz, 1H), 8.26 (d, *J*=8.0 Hz, 1H),7.78-7.86 (m, 2H), 7.49-7.64(m, 2H), 7.44-7.49(m, 2H), 7.35(s, 2H), 7.14(s, 1H), 5.62 (s, 2H, O-C***H***$_2$), 2.41 (s, 6H, -CH_3); ^{13}C-NMR(100 MHz, $CDCl_3$): δ_C 153.1, 143.6, 138.4, 131.4, 127.1, 126.7, 125.3, 123.6, 122.4, 121.3, 120.6, 119.8, 118.2, 61.5, 21.1; ESI-MS: 329[M+H]$^+$;Anal. Calcd for $C_{20}H_{16}N_4O$: C, 73.15; H, 4.91; N, 17.06. Found: C, 73.23; H, 4.97; N, 17.01.

3-(3,5-dichlorophenyl)-3,11-dihydro-[1,2,3]triazolo [4',5':4,5]pyrano [3,2-h]quinoline(3k): Yellow solid; IR (KBr, cm^{-1}) ν_{max}3087 (Ar-H), 1648 (C=N),1557(C=C), 1327 (C-Cl); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H8.88 (d, *J*=8.0 Hz, 1H), 8.23 (d, *J*=7..2 Hz, 1H), 7.89-8.02 (m, 1H), 7.77(s, 2H), 7.53-7.68(m, 2H), 7.37-7.50 (m, 1H), 5.65 (s, 2H, O-C***H***$_2$); ^{13}C-NMR(100 MHz, $CDCl_3$): δ_C 153.8, 144.2 138.9, 134.1, 132.7, 131.2, 126.8, 123.4, 122.3, 121.4, 118.7, 62.2; ESI-MS: 370[M+H]$^+$;Anal. Calcd for $C_{18}H_{10}Cl_2N_4O$: C, 58.56; H, 2.73; N, 15.18. Found: C, 58.48; H, 2.67; N, 15.11.

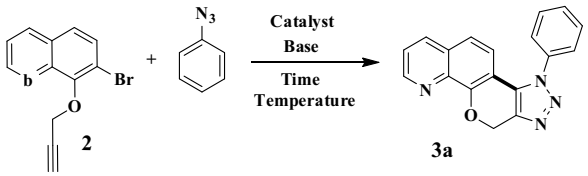
Cytotoxic activity

All of the synthesized compounds were evaluated for their in vitro cytotoxic activity against three different cancer cell lines such as MCF-7 (breast), A-549 (alveolar) and HeLa (cervical). All the cancer cell lines used in this research work was obtained from National Centre for Cell Sciences (NCCS), Pune, India. Cell viability in the presence of the test samples was measured using the MTT-micro culturedtetrazolium assay (Shekan et al., 1990; Monks et al., 1991). This assay is a quantitative colorimetric method for the determination of cell cytotoxicity. The assessed parameter is the metabolic activity of viable cells. Metabolically active cells reduce pale yellow tetrazolium salt (MTT) to a dark blue water-insoluble formazan, which can be directly quantified after solubilization with DMSO. The absorbance of the formazan directly correlates with the number of viable cells. Human cells were plated into a 96-well plate at a density of 1X10^4 cells per well. Cells were grown overnight in the full medium and then switched to the low serum media. DMSO was used as a control. After 48 h of treatment with different concentrations of test compounds, the cells were incubated with MTT (2.5 mg mL^{-1}) in the CO_2 chamber for2 h. The medium was then removed and 100 µL of DMSO was added into each well to dissolve the formazan crystals. After thoroughly mixing, the plates were read at 570 nm for optical density, which is directly correlated with cell quantity. The results were represented as a percentage of cytotoxicity/ viability. All of the experiments were carried out in triplicates. The IC_{50}values were calculated from the percentage of cytotoxicity and compared with the reference drug.

Results and discussion

The synthetic procedure adopted to obtain the target compounds is shown in **Scheme 1**. The intermediate 7-bromo-8-(prop-2-ynyloxy) quinoline (**2**) was prepared with high yields (86%) using 7-bromoquinolin-8-ol (**1**) and propargyl bromide in the presence of anhydrous K_2CO_3 in DMF.With the aim of preparing fused 1, 2, 3-triazoles, our

Table 1. Optimization of CuI-catalyzed intramolecularC-C coupling[a]



Entry	Catalyst (10 mol%)	Solvent	Base	Temp (^oC)	Time(h)	Yield[b](%)
1	CuI	THF	*t*-BuOK	80	20	17
2	CuI	DMSO	*t*-BuOK	80	20	20
3	CuI	DMF	*t*-BuOK	80	20	26
4	CuI	THF-H_2O	*t*-BuOK	80	20	16
5	CuI	DMF	*t*-BuOK	120	24	36
6	CuI	DMF	*t*-BuOLi	120	24	23
7	CuI	DMF	K_2PO_3	120	24	N.R
8	CuI	DMF	Cs_2CO_3	120	24	N.R
9	CuI	DMF	*t*-BuOK	140	24	58
10	**CuI**	**DMF**	***t*-BuOK**	**150W(MW)**	**15 min**	**78**
11	**CuI**	**DMF**	***t*-BuOK**	**200W(MW)**	**20 min**	**74**

[a]Reactions were performed with **2** (1.0 mmol), azide (1.0 mmol) and ^{t}BuOK (2.0 mmol); [b]Isolated yield; N.R. = No Reaction.

initial investigation began with the reaction of **2** and phenyl azide with 10mol % of CuI in THF at 80°C for 20 h. The desired product was formed in 17% yield (**Table 1**, entry 1). Similarly, the reaction was carried out using different solvents DMSO, DMF, and H_2O (**Table 1**, entries 2–4), the product was formed with yields not exceeding 27%, with DMF being the most favorable solvent (26% yield). Then, the reaction was carried out using 10 mol % of CuI at 120°C for 24 h, the desired product was obtained with 36% yield (**Table 1**, entry 5). When t-BuOK was replaced with t-BuOLi, K_2PO_3 and Cs_2CO_3, we could not get the desired products in good yield compared to t-BuOK (**Table 1**, entries 6–8). When the reaction was carried out at higher temperatures (above 140°C) using t-BuOK and DMF the product was obtained in 58% yield (**Table 1**, entry 9). Encouraged by these results the reaction was carried out under microwave irradiation, which resulted in the generation of the desired product in excellent yields (**Table 1**, entry 10, 11). After the complete optimization studies, it was clear that the use of catalyst load of CuI (10 mol %) with 2 equivalents of t-BuOK in DMF under the microwave irradiation (150 W) was the optimal reaction conditions to obtain the desired products in good yields. Several substituted phenyl azides containing electron-donating groups (methyl, and methoxy groups) and electron-withdrawing groups (chloro and nitro groups), were all compatible with this transformation under the optimal reaction conditions (**Figure 3**).

The structures of the newly synthesized compounds **3a-3k** were confirmed by spectral data (^{1}H NMR, ^{13}C NMR, IR, and ESI-MS) and elemental (CHN) analysis. All the spectral and analytical data of the synthesized compounds were in full agreement with the proposed structures and also discussed for a representative compound **3b**. From the IR spectrum, the appearance of a broad absorption band at sharp bands at 3074, 1638 and 1567 cm^{-1} are ascribed to Ar-H, C=N and -C=C stretching frequencies, respectively. From the ^{1}H NMR spectrum, the signals appearance at 3.86 (s, 3H, O-CH_3), 5.65 (s, 2H, O-CH_2), 7.02 to 8.75 (m, 9H, Ar-***H***), and from the ^{13}C NMR, the presence of signals at 158.1 ppm (***C***-OCH_3), 153.7 ppm (***C***-O-CH_2), 61.5 ppm (-O***C***H_2), 57.6 ppm (-O***C***H_3) and the ESI- mass spectra of **3b** showed [M+H] ion peak at m/z 331, which confirmed the structure of compound **3b.** The elemental analyses (CHN) data (C, 68.09; H, 4.19; N, 17.04*)* confirmed the purity of compound **3b.**

Anticancer activity

In vitro anticancer activity results revealed that all the compounds were activeagainst all the three tested cell lines such as breast carcinoma (MCF-7), alveolar carcinoma (A-549) and cervical carcinoma (HeLa). Cellviability in the presence of the test samples was measured using the MTT-microcultured tetrazolium assay. Doxorubicin was used as a positive control and the results are summarized in **table 2**.

Among them, the compound derived from the 3,5-dimethylphenygroup on fused triazole ring i.e. **3j** has shown

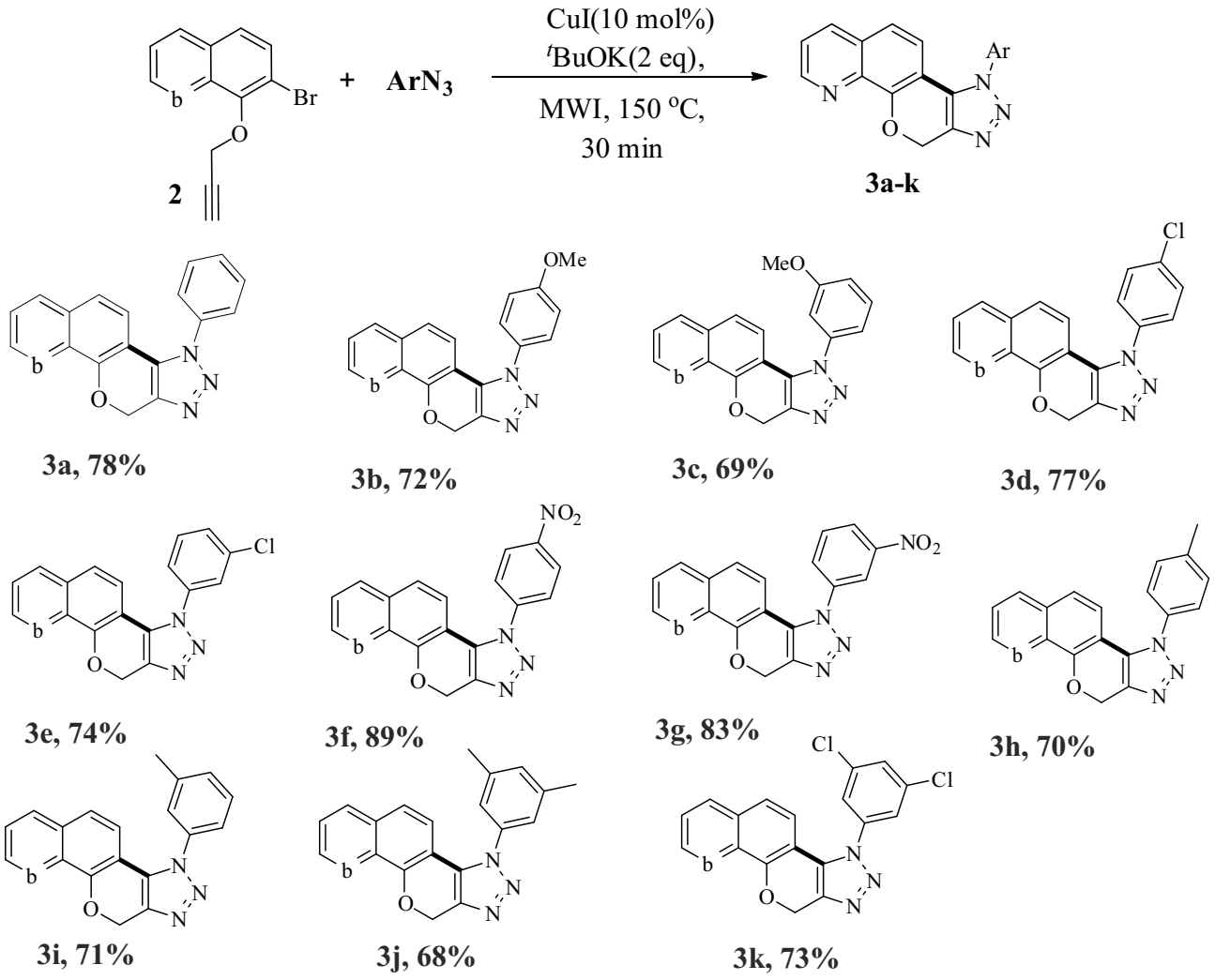


Figure 3. Cu/Pd catalyzed one pot microwave synthesis of fused [1,2,3]triazolyl[4',5':4,5] pyrano[3,2-h]quinolines. The isolated yields are given as percentages

Table 2. Cytotoxic activity of fused 1,2,3-triazoles (**3a-3k**) on MCF-7, A-549 and HeLa [*in vitro*[a] (IC_{50}µM][b]

Product	MCF-7	A-549	HeLa
3a	41.86 ± 0.7	62.88± 1.2	46.39±1.0
3b	89.21± 0.9	77.52± 1.3	**24.92± 0.8**
3c	**31.44± 0.5**	44.52± 1.1	**15.67± 0.6**
3d	47.67± 0.8	59.62± 1.1	49.14± 1.0
3e	55.27± 1.4	75.89± 1.6	54.57± 0.9
3f	79.88± 1.3	96.87± 1.5	68.21± 1.1
3g	81.37± 1.6	69.54± 1.0	51.79± 0.8
3h	39.18± 1.0	42.97± 1.3	**21.27± 0.7**
3i	40.66± 0.8	63.47± 1.1	**31.66± 0.9**
3j	**16.88± 0.5**	**32.47± 1.0**	**11.42± 0.6**
3k	49.55± 1.0	67.88± 1.4	43.68± 1.1
Doxorubicin	**3.01 ± 0.2**	**1.08 ± 0.08**	**1.35 ± 0.04**

[a]Values are expressed as mean ± SEM. [b]Cytotoxicity,

potent activity against MCF-7 & HeLa with IC_{50} values 16.88 ± 0.5 & 11.42 ± 0.6μM and moderate activity against A-549 with IC_{50} value 32.47 ± 1.0μM respectively. Compound **3c**, having a 3-methoxyphenyl group on triazole ring exhibited potent activity against HeLa with IC_{50} value 15.67 ± 0.6μM and moderate activity against the MCF-7 cell line with an IC_{50} value of 31.44 ± 0.5μM. These results are comparable to those of the standard drug doxorubicin. Similarly, the introduction of 4-methoxyphenyl group and 4-methylphenyl group on fused triazole ring i.e. **3b** and **3h** have shown good activity against HeLa cell line with IC_{50} values 24.92 ± 0.8 & 21.27 ± 0.7μM respectively. Remaining compounds have shown moderate activity with IC_{50} values ranging from 39.18± 1.0 -96.87± 1.5μM against the three cell lines. It is necessary to point out that all of the potent analogues contain methyl, dimethyl and methoxy substituents on the triazole ring (3b, 3c, 3h, 3i & 3j) and this preference was uniform and irrespective to the substitution pattern on the aromatic ring. The electron withdrawing substituents such as chloro and nitro groups on the triazole ring exhibited moderate to poor activity against all three tested cell lines.

Conclusion

In summary, we have developed a new method for the synthesis of [1,2,3] triazolyl [4 ', 5': 4,5] pyrano [3,2-h] quinoline derivatives. The protocol involves a Cu-catalyzed one pot [3+2] cyclo addition followed by C-C bond coupling reaction under microwave condition without isolating the intermediate 1,4-disubstituted 1,2,3-triazole. These new compounds were tested for their in vitro cytotoxic activity against the cancer cell lines MCF-7, A-549 and HeLa. Among them, compounds **3c** and **3j** showed strong activity against HeLa. Interestingly, most of the analogues showed strong activity against HeLa and moderate activity against MCF-7 and A-549. In general, the results indicate that these compounds have the potential to develop as leads, and their additional simple structural modifications in the title compounds can produce promising anti-cancer agents for the human cervical cancer HeLa cell line.

Acknowledgments

The authors are thankful to the Director of Indian Institute of Chemical Technology in Hyderabad for recording ^{1}H, ^{13}C NMR and mass spectra. The authors are thankful to the Head, Department of Bio-Technology, Kakatiya University, and Warangal for providing data of biological activity.

Conflicts of interest

The authors declare no conflict of interest.

References

Ackermann L, Althammer A, Fenner S. 2009. Palladium-Catalyzed direct arylations of heteroarenes with tosylates and mesylates. Angewandte Chemie International Edition 48: 201-204.

Ackermann L, Vicente R, Born R. 2008.Palladium-Catalyzed Direct Arylations of 1,2,3-Triazoles with Aryl Chlorides using Conventional Heating. Advanced Synthesis & Catalysis 350: 741-748.

Ackermann L, Rajkumar J, Harish K P, Novak P, Buttner L. 2010.Palladium-CatalyzedDehydrogenative Direct Arylations of 1,2,3-Triazoles.Organic Letters 12: 2056-2059.

Alvarez R, Velazquez S, San-Felix A, Stefano A, Clercq E D, Carlo-Federico P, Karlsson A,Jan B, Camarasa M J. 1994. 1,2,3-Triazole-[2 ',5'-Bis-O(-t ert-butyldimethylsilyl)- -D-ribofuranosyll-3'-spiro-5”-(4'-amino-l”,2”-oxathio2l”e, 2-dioxide)(TSAO)A nalogues: Synthesis and Anti-HIV-1 Activity. Journal of Medicinal Chemistry 37: 4185–4194.

Angell Y L, Kevin Burgess. 2007. Peptidomimetics via copper-catalyzed azide–alkynecycloadditions. Chemical Society Reviews 36: 1674–1689.

Atwell G J, Baguley B C, Denny W A. 1989.Potential antitumor agents. 57. 2-Phenylquinoline-8-carboxamides as minimal DNA-intercalating antitumor agents with in vivo solid tumor activity. Journal of Medicinal Chemistry 32: 396-401.

Chung-Yu C, Cheng-Hao Y, Wan-Ping H, Jaya K, Vandavasi, Mei-Ing C, Jeh-Jeng W. 2013. Synthesis of fused triazolo[4,5-d]quinoline/chromene/ thiochromene derivatives via palladium catalysis mediated by tetrabutylammonium iodide. RSC Advances 3: 2710-2719.

Desai N C, Rajpara K M, Joshi V V. 2012. Synthesis and characterization of some newquinoline based derivatives endowed with broad spectrum antimicrobial potency. Bioorganic and Medicinal Chemistry Letters 22: 6871-6875.

Fournier D, Hoogenboom R, Schubert US. 2007.Clicking polymers: a straightforwardapproach to novel macromolecular architectures. Chemical Society Reviews 36: 1369–1380.

Harjinder S, Bhaskara N, Jayant S, Jitender MK, Chetan S, Kamal RA. 2014.Efficient OnePot Synthesis of Xanthene-Triazole-Quinoline/Phenyl Conjugates and Evaluation of their Antimicrobial Activity. Journal of the Brazilian Chemical Society 25: 1178-1193.

Hartmuth CK, Sharpless KB. 2003. The growing impact of click chemistry on drugdiscovery. Drug Discovery Today 8: 1128–1137.

Insuasty B, Montoya A, Becerra D, Quiroga J, Abonia R, Robledo S, Vélez ID, Upegui Y, Nogueras M, Cobo J. 2013. Synthesis of novel analogs of 2-pyrazoline obtained from [(7-chloroquinolin-4-yl)amino]chalcones

and hydrazine as potential antitumor and antimalarial agents. European Journal of Medicinal Chemistry 67: 252-262.

Kaur K, Jain M, Reddy RP, Jain R. 2010.Quinolines and structurally related heterocyclesas antimalarials. European Journal of Medicinal Chemistry 45: 3245-3264.

Kishna RS, Pankaj S, Srinivasa RT, Manish KJ, Lakshma NV, Naidu VGM, Ahmed K, Shankaraiah N. 2015. Spirooxindole-derived morpholine-fused-1,2,3-triazoles:Design, synthesis, cytotoxicity and apoptosis inducing studies. European Journal of Medicinal Chemistry 102: 413-424.

Michael JG, Holly H, Ashraf B, Ying-Chuan L, Gabrielle C, Chi-Huey W, Duncan EM, John HE, David CS, Bruce ET. 2008. A Copper (I)-Catalyzed 1,2,3-Triazole Azide-Alkyne Click Compound is a Potent Inhibitor of a Multidrug-Resistant HIV-1 Protease Variant. Journal of Medicinal Chemistry 51:6263–6270.

Michael JP. 2007. Quinoline, quinazoline and acridone alkaloids. Natural Product Reports 24: 223-246.

Monks A, Scudiero D, Shekan P, Shoemaker R, Pamil K, Vistica D, Hose C, Langley J, Cromise P. 1991. Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines. Journal of the National Cancer Institute 83:757–766.

Musiol R, Jampilek J, Kralova K, Richardson D R, Kalinowski D, Podeszwa B, Finster J, Niedbala H, Palka A, Polanski J. 2007. Investigating biological activity spectrum for novel quinoline analogues. Bioorganic and Medicinal Chemistry 15: 1280-1288.

Musiol R, Serda M, Hensel-Bielowka S, Polanski J. 2010. Quinoline-basedantifungals.Current Medicinal Chemistry 17:1960-1973.

Narsimha S, Kumara Swamy B, Satheesh KN, Ramesh G, Narasimha RY, Vasudeva RN. 2016a. One-pot synthesis of fused benzoxazino[1,2,3]triazolyl[4,5-c]quinolinone derivatives and their anticancer activity. RSC Advances 6: 74332–74339.

Narsimha S, Kumar NS, Kumaraswamy B, Vasudeva RN, Hussain AS, SrinivasaRao M. 2016b. Indole-2-carboxylic acid derived mono and bis 1,4-disubstituted 1,2,3-triazoles: synthesis, characterization and evaluation of anticancer, antibacterial, and DNA cleavage activities. Bioorganic and Medicinal Chemistry Letters 26:1639–1644.

Narsimha S, Kumara SB, Reddy NV. 2018a. One-pot synthesis of novel 1,2,3-triazolepyrimido[4,5-c]isoquinoline hybrids and evaluation of their antioxidant activity. Synthetic Communications 48:1220-1226.

Narsimha S, Kumara SB, Yellu NR, Reddy NV. 2018b. Microwave-assisted Cu-catalyzed C–C bond formation: one-pot synthesis of fully substituted 1,2,3-triazoles using nonsymmetrical iodoalkynes and their biological evaluation. Chemistry of Heterocyclic Compounds 54(12): 1161–1167.

Narsimha S, Kumar TR, Kumar NS, Yakub S, Vasudeva RN. 2014. Synthesis andantibacterial activity of (1-aryl-1, 2, 3-triazol-4-yl) methyl esters of morpholine-3-carboxylic acid. Medicinal Chemistry Research 23:5321–5327.

Panteleev J, Geyer K, Angelica AA, Wang L, Lautens M. 2010. C-H Bond Functionalizationin the Synthesis of Fused 1,2,3-Triazoles. Organic Letters12: 5092-5095.

Rajkumar J, Harish KP, Ackermann L. 2012. Copper-catalyzed CuAAC/intramolecular C–H arylation sequence: Synthesis of annulated 1,2,3-triazoles. Beilstein Journal of Organic Chemistry 8: 1771–1777.

Ranjith KT, Narsimha S, Swamy BK, Chary VR, Estari M, Reddy NV. 2017. Synthesis, anticancer and antibacterial evaluation of novel (isopropylidene) uridine-[1,2,3]triazole hybrids. Journal of Saudi Chemical Society 21: 795-802.

Shekan P, Storeng R, Scudiero D, Monks A, Mc Mohan J, Vistica D, Warren JT, Bokesch H,Kenncy S, Boyd MR. 1990.New colorimetric cytotoxicity assay for anticancer-drug screening. Journal of the National Cancer Institute 82:1107–1112.

Sheng-Jiao Y, Yong-Jiang L, Yu-Lan C, Lin L, Jun L. 2010.An efficient one-pot synthesis of heterocycle-fused 1,2,3-triazole derivatives as anti-cancer agents. Bioorganic and Medicinal Chemistry Letters 20: 5225–5228.

Sumangala V, Boja P, Chidananda N, Jennifer F, SuchethaKumari N. 2010. Synthesis andAntimicrobial Activity of 1,2,3-Triazoles Containing Quinoline Moiety. Archives of Pharmacal Research 33: 1911-1918.

Swamy BK, Narsimha S, Kumar TR, Reddy YN, Reddy NV. 2017. Synthesis and Biological Evaluation of Novel Thiomorpholine 1,1-Dioxide Derived 1,2,3-Triazole Hybrids as Potential Anticancer Agents. Chemistry Select 2: 4001-4005.

Swamy BK, Narsimha S, Kumar TR, Reddy YN, Reddy NV. 2017a. Synthesis and Biological Evaluation of (N-(3-methoxyphenyl)-4-((aryl-1H-1,2,3-triazol-4-yl)methyl) thiomorpholine 2-carboxamide 1,1-Dioxide Hybrids as Antiproliferative Agents. Chemistry Select 2: 9595–9598.

Swamy BK, Narsimha S, Reddy NV, Priyanka B, Rao MS. 2016. Synthesis and antimicrobial evaluation of some

novel thiomorpholine derived 1,4-disubstituted 1,2,3-triazoles. Journal of the Serbian Chemical Society 81: 233-242.

Thomas KD, Airody VA, Suchetha SN. 2010. Design, synthesis and antimicrobial activities of some new quinoline derivatives carrying 1,2,3-triazole moiety. European Journal of Medicinal Chemistry 45: 3803-3810.

Thomas KD, Airody V A, Imran H. C. Sumesh E, Nishith K P. 2011. New quinolin-4-yl-1,2,3- triazoles carrying amides, sulphonamides and amidopiperazines as potential antitubercular agents. European Journal of Medicinal Chemistry 46: 2503-2512.

Tienan J, Shin K, Yoshinori Y. 2004. Copper-Catalyzed Synthesis of N-Unsubstituted 1,2,3-Triazoles from Nonactivated Terminal Alkynes. European Journal of Organic Chemistry 18: 3789–3791.

Vasudeva RN, Narsimha S, Kumaraswamy B, Sudhakar L, Hussain AS. 2016a. N,N'-(hexane-1,6-diyl)bis(N-((1-aryl/alkyl-1H-1,2,3-triazol-4-yl)methyl)-4-methyl benzene sulfon -amide): synthesis, antibacterial, antioxidant, and DNA-cleavage activities. Phosphorus Sulfur Silicon Relat Elements 191, 1118-1122.

Vasudeva RN, Satheesh KN, Narsimha S, Kumara SB, Savitha JT, Narasimha YR. 2016b. Synthesis, characterization and biological evaluation of 7-substituted- 4-((1-aryl-1H-1,2,3-triazol-4-yl) methyl)-2H-benzo[b][1,4]oxazin- 3(4H)-ones as anticancer agents. Medicinal Chemistry Research 25:1781-1793.