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Solvent effect on copper-catalyzed azide–alkyne cycloaddition (CuAAC): Synthesis of novel triazolyl substituted quinolines as potential anticancer agents

Amarender Reddy Ellanki [a,e], Aminul Islam [a], Veera Swamy Rama [a], Ranga Prasad Pulipati [a], D. Rambabu [b,c], G. Rama Krishna [d], C. Malla Reddy [d], K. Mukkanti [e], G.R. Vanaja [f], Arunasree M. Kalle [f], K. Shiva Kumar [b], Manojit Pal [b,*]

[a] Chemistry-Technology Development Center, Dr. Reddy's Laboratories Limited, Bollaram Road, Miyapur, Hyderabad 500049, Andhra Pradesh, India
[b] Institute of Life Sciences, University of Hyderabad Campus, Gachibowli, Hyderabad 500046, India
[c] Department of Chemistry, K. L. University, Vaddeswaram, Guntur 522502, Andhra Pradesh, India
[d] Department of Chemical Sciences, Indian Institute of Science Education and Research, Kolkata, West Bengal 741252, India
[e] Chemistry Division, Institute of Science and Technology, JNT University, Kukutpally, Hyderabad 500072, Andhra Pradesh, India
[f] Department of Animal Sciences, School of Life Sciences, University of Hyderabad, Hyderabad 500046, India

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ABSTRACT

A regioselective route to novel mono triazolyl substituted quinolines has been developed via copper-catalyzed azide–alkyne cycloaddition (CuAAC) of 2,4-diazidoquinoline with terminal alkynes in DMF. The reaction provided bis triazolyl substituted quinolines when performed in water in the presence of Et_3N. A number of the compounds synthesized showed promising anti-proliferative properties when tested in vitro especially against breast cancer cells.

Nitrogen hetrocycles play a central role both in medicinal chemistry and drug discovery. The development of newer synthetic methods useful for the assembly of molecules containing nitrogen heterocycles therefore has attracted particular attention both in academia and industrial organizations. Quinoline derivatives that belong to this class have shown wide applications as drugs and pharmaceuticals[1–6] in addition to their widespread occurrences in natural products for example, Camptothecin[2] and Luotonin A.[3] Quinoline fused with tetrazole has also exhibited notable pharmacological properties for example, 4-(1*H*-tetrazol-5-yl)tetrazolo[1,5-*a*]quinoline (**A**, Fig. 1) and 4-argio-6-(tetrazolo[1,5-*a*]quinolin-4-yl)-5,6-dihydropyrimidin-2(1*H*)-one has shown antiallergic[7a] and anti-inflammatory activities,[7b] respectively. The 4-triazolyl quinoline derivatives on the other hand have been reported as inhibitors of human tumor cells growth.[8] Due to our longstanding interest in the synthesis of quinoline derivatives[9] of potential pharmacological significance we choose to prepare novel triazolyl substituted quinoline derivatives for assessing their anticancer properties in vitro. Herein we report our initial findings on

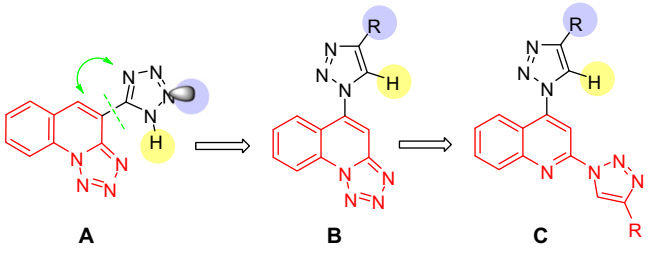
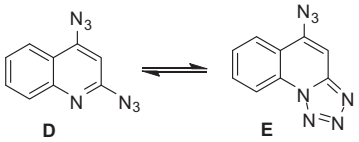


Figure 1. General structure of proposed small molecule library (**B** and **C**) derived from **A**.



Scheme 1. Tautomeric forms of 2,4-diazidoquinoline.

* Corresponding author. Tel.: +91 40 6657 1500; fax: +91 40 6657 1581.
E-mail address: manojitpal@rediffmail.com (M. Pal).

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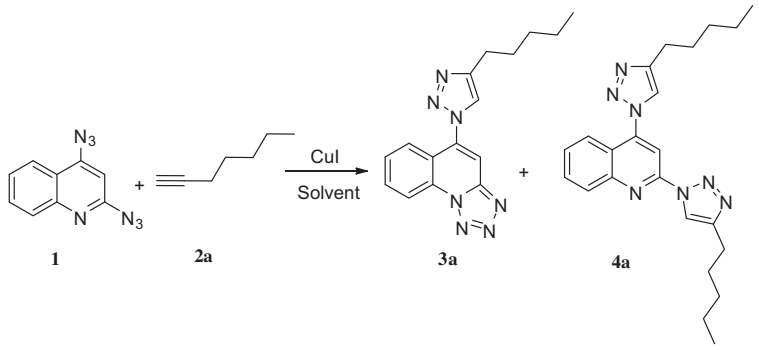
Table 1
Optimization of the reaction conditions[a]



Entry	Cu catalyst	Solvent	Time (h)	Yield (%)	
				3a	**4a**
1	CuI	DMSO	12	75	10
2	CuI	DMF	10	85	n.d.
3	CuI	DMF–H_2O (1:1)	10	50	30
4	CuI	H_2O	12	n.d.	60[b]
5	CuI	H_2O	12	n.d.	89[b,c]
6	Sodium ascorbate–$CuSO_4{\cdot}5H_2O$	*t*-BuOH/H_2O (1:1)	20	n.d.	n.d.

n.d. = Not detected.
[a] All the reactions were carried out by using compound **1** (1.0 equiv), hept-1-yne **2a** (1.5 equiv), and CuI (0.01 equiv) at 60–70 °C.
[b] The reaction was carried out using 3.0 equiv of Et_3N.
[c] The reaction was carried out using 3.0 equiv of **2a**.

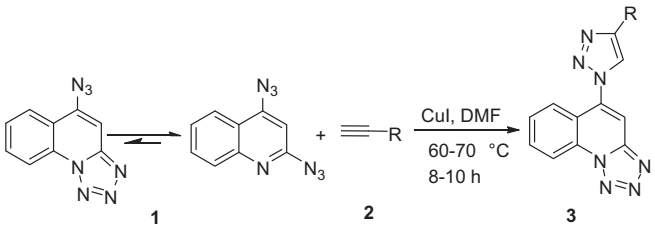
the synthesis and pharmacological evaluation of a series of compound **B** and **C** related to **A** as potential anti-cancer agents (Fig. 1). The substituent 'R' was introduced to generate a library of small molecules.

Since the report of click chemistry[10a] by Kolb, Finn, and Sharpless in 2001 there has been an explosive growth in this area of chemistry. The copper-catalyzed azide–alkyne cycloaddition (CuAAC)[10b,c] on the other hand represents the best paradigm of click chemistry. Traditionally, the construction of triazole ring via Cu(I)-catalyzed 1,3-cycloaddition of an azide to a terminal alkyne generally involves the use of a mixture of $CuSO_4$ and sodium ascorbate as a precatalyst system which generates the Cu(I) species in situ in the reaction mixture. Recently, the use of a number of alternative Cu-salts for the generation of Cu(I) species in situ have been reported which include $Cu(OTf)_2$,[11] Cu_2O,[12] CuBr,[13] $[(ICy)_2Cu]PF_6$,[14] tris(triazolyl)methanol-Cu(I) structure[15] etc. Although all these approaches have their own merits and synthetic values some of them however suffer from few disadvantages for example, the use of expensive catalysts and ligands and utilization of harsh reaction conditions. Moreover, the microwave assisted synthesis of 3-triazolyl-2(1*H*)-quinoline and 4-triazolyl-2(1*H*)-quinolines that involved the 1,3-dipolar cycloaddition of organic azides and terminal acetylene in the presence of $CuSO_4{\cdot}5H_2O$, sodium ascorbate in DMF at 110 °C for 30 min has been reported.[16a] While the use of CuI in click chemistry has been explored earlier its use in the preparation of triazolyl substituted quinoline derivatives has not been reported.[16b] Herein we report CuI-mediated efficient and mild synthesis of novel triazolyl substituted quinoline derivatives without using ascorbic acid or its salt.

For the generation of quinoline based small molecule library 2,4-diazidoquinoline was chosen as the starting compound that is known to exist in two tautomeric forms for example, 2,4-diazidoquinoline (**D**) and 5-azidotetrazolo[1,5-*a*]quinoline (**E**) (Scheme 1). The equilibrium between these two tautomeric forms[17–19] depend on several factors, such as the nature of substituents present,[17] solvent,[18] and temperature.[17] Nevertheless, the reaction of 2,4-diazidoquinoline[19] (**1**) with hept-1-yne (**2a**), was examined initially to establish the optimized reaction conditions and the results are summarized in Table 1. When the compound **1** (1.0 equiv) and alkyne **2a** (1.5 equiv) were reacted in DMSO in the presence of CuI at 60–70 °C for 12 h a mixture of products that is, mono triazolyl substituted compound (**3a**) as major and bis triazolyl substituted compound (**4a**) as minor product were obtained (Table 1, entry 1). However, changing the solvent to DMF increased the yield of **3a** to 85% (Table 1, entry 2) and suppressed the formation of **4a**. While the use of DMF–H_2O provided almost 1:1 mixture of **3a** and **4a** (Table 1, entry 3) the compound **4a** was isolated as the only product in water in the presence of Et_3N (Table 1, entry 4). The yield of **4a** was improved when 3.0 equiv of alkyne **2a** was used (Table 1, entry 5). These observations suggested that the compound **1** existed predominantly in form **E** (Scheme 1) in DMF leading to the mono triazolyl substituted product **3a**. The protic solvent water in presence of Et_3N favored the form **D** (perhaps via H-bonding) thereby allowing compound **1** to yield bis triazolyl substituted product **4a**. Nevertheless, while CuI was used as a catalyst in all these reactions the use of other catalysts for example, the combination of sodium ascorbate–$CuSO_4{\cdot}5H_2O$ was also examined in *t*-BuOH/H_2O (1:1) but found to be ineffective under the condition employed (Table 1, entry 6). Additionally, the CuI mediated reaction in H_2O/tBuOH (1:1) provided a mixture of mono-triazole **3a** and bis-triazole **4a**. Thus, the best reaction condition for the preparation of **3a** without generating any side products was found to be CuI in DMF at 60–70 °C.

Having the optimized reaction condition for the preparation of mono triazolyl substituted compound in hand we then examined the generality and scope of this methodology. Thus a variety of terminal alkynes (**2**) were reacted with 2,4-diazidoquinoline (**1**) in the presence of CuI in DMF at 60–70 °C and results are presented in Table 2. The reaction proceeded well in all these cases and the substituents like aryl, alkyl, hydroxy alkyl or chloro present in the alkyne were well tolerated. The formation of no side product was observed and the desired product **3** was isolated in good yield in each case. All the compounds synthesized were well characterized by spectral (NMR, MS and IR) data. Additionally, the molecular

Table 2
Synthesis of triazolyl substituted tetrazolo quinoline (**3**) via Cu mediated click reaction between 2,4-diazidoquinoline and different alkynes[a]



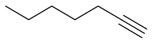
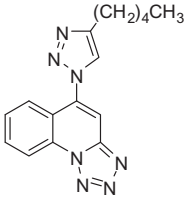
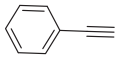
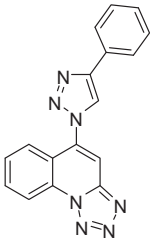
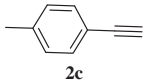
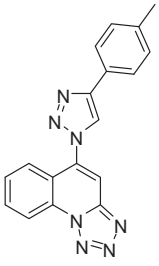
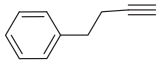
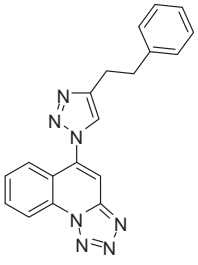
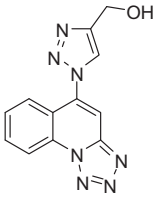
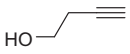
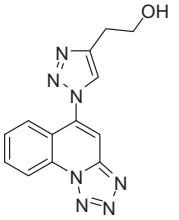
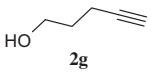
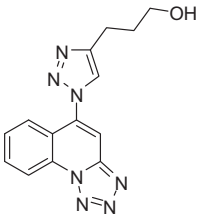
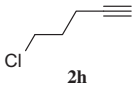
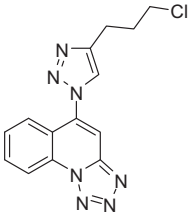
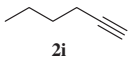
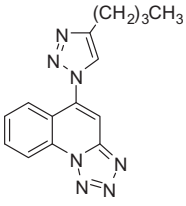
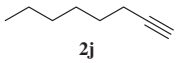
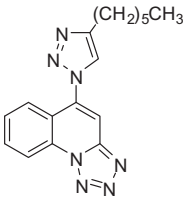
Entry	Alkynes (**2**)	Products[b] (**3**)	Yield[c] (%)
1	 **2a**	 **3a**	85
2	 **2b**	 **3b**	82
3	 **2c**	 **3c**	88
4	 **2d**	 **3d**	83
5	 **2e**	 **3e**	78

Table 2 (*continued*)

Entry	Alkynes (**2**)	Products[b] (**3**)	Yield[c] (%)
6	 **2f**	 **3f**	86
7	 **2g**	 **3g**	85
8	 **2h**	 **3h**	80
9	 **2i**	 **3i**	85
10	 **2j**	 **3j**	87

[a] All the reactions were carried out by using compound **1** (1.0 equiv), terminal alkyne **2** (1.5 equiv), and CuI (0.01 equiv) at 60–70 °C.
[b] Identified by ^{1}H NMR, IR and MS.
[c] Isolated yields.

structure of a representative compound **3d** was established unambiguously by single crystal X-ray diffraction (Fig. 2)[20] which confirmed the presence of a fused tetrazole moiety.

Since bis triazolyl substituted compound (**4a**) was obtained exclusively when the reaction was performed in water in the presence of Et_3N and 3.0 equiv of terminal alkyne (Table 1, entry 5) hence we examined the further scope of this methodology. Thus a number of unprecedented bis triazolyl derivatives[21] (**4**) were prepared in good yields by using this methodology (Table 3). While the role of Et_3N in the present reaction was not clearly understood,

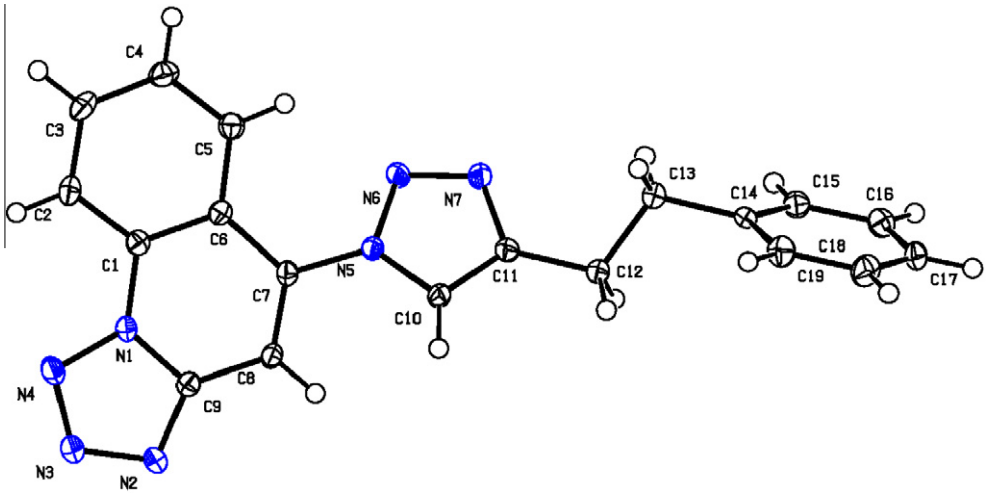
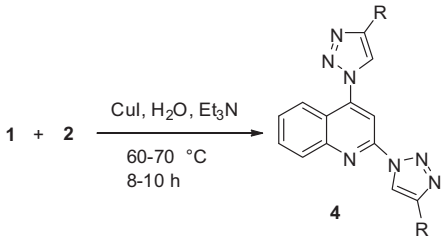


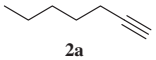
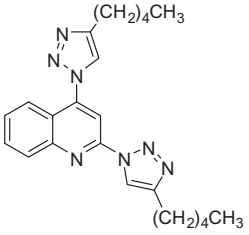
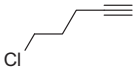
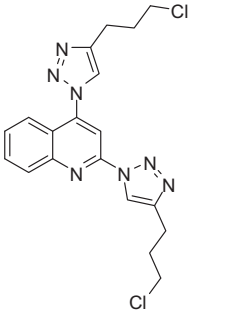
Figure 2. ORTEP representation of the compound **3d** (Thermal ellipsoids are drawn at 50% probability level).

the formation of bis-triazolyl derivatives however seemed to proceed via stepwise formation of mono-triazole ring initially followed by the bis-triazole ring. Once formed the mono-triazolyl derivative would be less soluble in water due to the presence of hydrophobic side chain. However, the presence of Et_3N perhaps increased its solubility in water thereby facilitating the second step. Thus, the role of Et_3N is possibly twofold (i) it helped in generating the Cu-acetylide intermediate which is well known in the literature (ii) it increased miscibility of the reactants with water.

Many of the triazolyl substituted tetrazolo quinolines (**3**) synthesized were tested for their anticancer properties in vitro. Cancer is the second leading cause of death worldwide after cardiovascular diseases, according to WHO.[22] We were particularly interested in evaluating our compounds against leukemia (a type of cancer of the blood or bone marrow characterized by an abnormal increase of white blood cells) and breast cancer due to the growing incidences of these types of cancers all over the world. The test compounds were examined at two different concentrations in a MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay[23] and results are summarized in Table 4. Harmine, a member of β-carboline family of compounds showed cytotoxicity against HL60 and K562 cell lines[24] was used as a reference compound. While most of the compounds showed reasonable inhibition of human chronic myeloid leukemia cell growth at 10 μM (Table 4, entries 1–8) the compound **3g** however was found to be active (~47% at 1 μM) when tested at lower concentrations (Table 4, entry 5). On the other hand most of the tested compounds were found to be active against breast cancer cell lines across all the concentrations and **3h** was found to be the most potent (~60% at 1 μM) among them. In a dose response study, when tested at 10, 1, 0.5, 0.1 and 0.01 μM compound **3g** showed 52%, 47%, 30%, 22% and 9% inhibition against K562 cells (IC_{50} ~4.6 μM) whereas 70%, 60%, 46%, 24% and 11% inhibition was observed for compound **3h** against MDA-MB231 cells (IC_{50} ~0.8 μM). Notably, Harmine showed IC_{50} value of 45 and 54 μM when tested against K562 and MDA-MB231 cells respectively. Thus, compound **3g** and **3h** were found to be promising. In general, an aryl side chain directly attached to the triazole moiety of **3** (e.g. **3b** and **3c** vs **3d**) was found to be marginally less potent than others whereas a haloalkyl or hydroxy alkyl long chain (e.g. **3g** and **3h**) was found to be superior than a simple alkyl group (e.g. **3i** and **3j**). We also examined the bis triazolyl substituted quinolines (**4**) for their potential cytotoxicities against K562 and MDA-MB231 cells. However, none of them showed inhibition >30% when tested at 10 μM. Nevertheless, our studies clearly suggest that triazolyl substituted tetrazolo quinoline moiety could be an attractive template for the identification of novel and potential anticancer agents. Since breast cancer caused 458,503 deaths worldwide (13.7% of cancer deaths in women) in 2008 hence there is a need for the identification of new

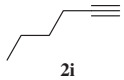
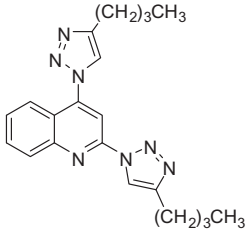
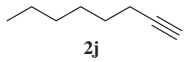
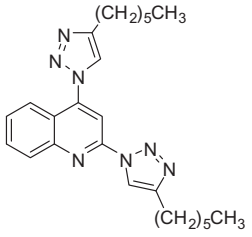
Table 3
Synthesis of bis triazolyl substituted quinoline (**4**) via CuI-mediated click reaction between 2,4-diazidoquinoline (**1**) and different alkynes[a]



Entry	Alkynes (**2**)	Products[b] (**4**)	Yield[c] (%)
1	 **2a**	 **4a**	89
2	 **2h**	 **4b**	78

[a] Reaction conditions: 1 (1 mmol), 2 (1 mmol), CuI (10 mol %), Et_3N (1 mmol), H_2O (5 mL), 60–70 °C, 8–10 h.
[b] Products were characterized by ^1H NMR, ^{13}C NMR, IR and mass spectral data.
[c] Isolated yields.

Table 3 (*continued*)

Entry	Alkynes (**2**)	Products[b] (**4**)	Yield[c] (%)
3	 **2i**	 **4c**	91
4	 **2j**	 **4d**	79

[a] All the reactions were carried out by using compound **1** (1.0 equiv), terminal alkyne **2** (1.5 equiv), Et_3N (3.0 equiv) and CuI (0.01 equiv) at 60–70 °C.
[b] Identified by ^1H NMR, IR and MS.
[c] Isolated yields.

Table 4
The %inhibition of growth of cancer cell lines by triazolyl substituted tetrazolo quinoline derivatives (**3**)[a]

Entry	Compounds	K562 (leukemia)		MDA-MB231 (breast)	
		10 μM	1 μM	10 μM	1 μM
1	**3b**	29.9	20.6	56.0	52.5
2	**3c**	44.1	20.6	54.0	47.7
3	**3d**	38.1	26.7	60.8	50.5
4	**3e**	36.9	21.8	46.8	45.8
5	**3g**	52.4	47.1	46.1	42.0
6	**3h**	35.2	19.9	70.1	60.2
7	**3i**	34.1	28.2	57.1	55.3
8	**3j**	37.4	19.1	53.4	49.7

[a] Data presented are average of three experiments.

agents to treat this deadly disease. The triazolyl substituted tetrazolo quinolines presented here therefore have medicinal value.

In conclusion, we have developed a regioselective method leading to mono triazolyl substituted quinolines via copper-catalyzed azide–alkyne cycloaddition (CuAAC) of 2,4-diazidoquinoline with terminal alkynes in DMF. CuI was identified as an effective catalyst for this purpose and the methodology does not require the use of ascorbic acid or its salt. The use of water in presence of Et_3N provided bis triazolyl substituted quinolines in good yields. A number of compounds showed promising anti-proliferative properties when tested against breast cancer cells in vitro. The triazolyl substituted tetrazolo quinoline moiety therefore could be an attractive template for the identification of novel and potential anticancer agents. The simple and general methodology presented here would find application in the preparation of diversity based library of small molecules related to triazolyl substituted quinoline of potential pharmacological interest.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.03.091.

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