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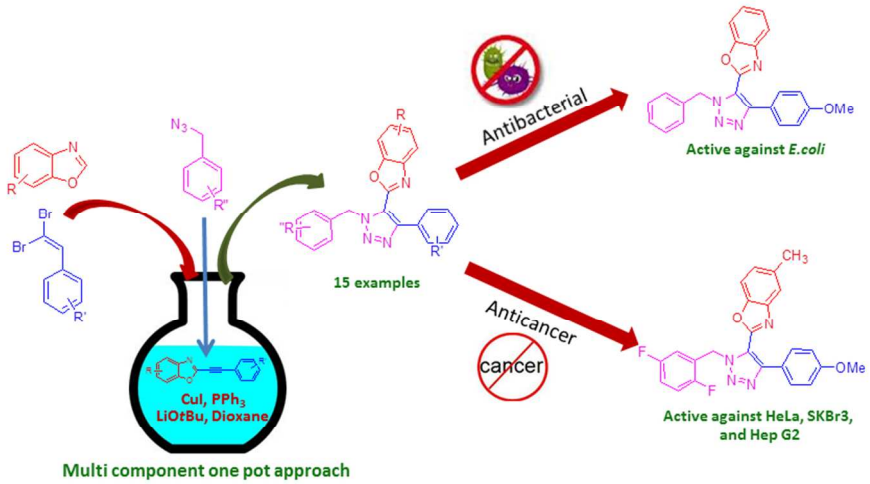

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One-pot sequential alkynylation and cycloaddition: Regioselective construction and biological evaluation of novel benzoxazole-triazole derivatives

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KEYWORDS: copper(I) iodide, C-H activation, cycloaddition, MCOP (multicomponent one-pot), cytotoxic

ABSTRACT: Individually, benzoxazole and triazole moieties are of significant biological interest owing to their importance in drugs and pharmaceuticals. To assess their combined biological impact when woven into one molecule, we designed a novel, regioselective, multi component, one pot (MCOP) approach for the construction of benzoxazole-linked triazoles. The synthesis has been achieved in two sequential steps involving copper catalyzed alkynylation of benzoxazole followed by a 1, 3-dipolar cycloaddition reaction. By combining these two bioactive units into one core, a series of new benzoxazole-triazole scaffolds has been synthesized and subjected to *in vitro* antibacterial and anticancer evaluation. Tests against clinical isolates of *Staphylococcus aureus* and *Escherichia coli* showed potent gram-negative activity for

compounds **4{*1,1,1*}**, **4{*1,1,4*}**, and **4{*1,2,1*}**. The cytotoxicity of the synthesized library was determined against three cancer cell lines: HeLa, SKBr3, and Hep G2. Compound **4{*2,2,2*}** showed significant cytotoxicity against all the cell lines. These preliminary bioassay evaluations strongly suggest the promise and scope of these novel molecules as therapeutic agents in medical science.

INTRODUCTION

A triazole possessing three nitrogen atoms in a five membered heterocyclic ring is an important pharmacophore and shows various clinical properties such as: anti-HIV,[1,2] antimicrobial,[3-7] antiparasitic,[8] anti-inflammatory,[9] anticancer,[10,11] antimalarial,[12] and antiviral.[13] Another clinically important substructure is benzoxazole, which is widely used as a starting material for the synthesis of bioactive structures and forms a part of important drugs such as flunoxaprofen and zoxazolamine. The benzoxazole derivatives have been investigated for their inhibitory activity on eukaryotic DNA topoisomerase,[14] and are known to exhibit antibacterial,[15,16] antitubercular,[17] anticancer,[18] antiparasitic,[19] and anti-HIV[20] activity (**Figure 1**). In view of such therapeutic significance of these compounds, several methods targeting their individual synthesis as well synthesis in combined form have been reported.[21-25] These methods, however, suffer from drawbacks of being carried out in multiple steps; generate a large number of byproducts, long reaction time, use of harsh reagents and conditions, and low overall yield of the final product. With a view of harnessing the interesting biological properties of triazole and benzoxazole molecules, we attempted designing a simple synthetic route to construct these scaffolds.

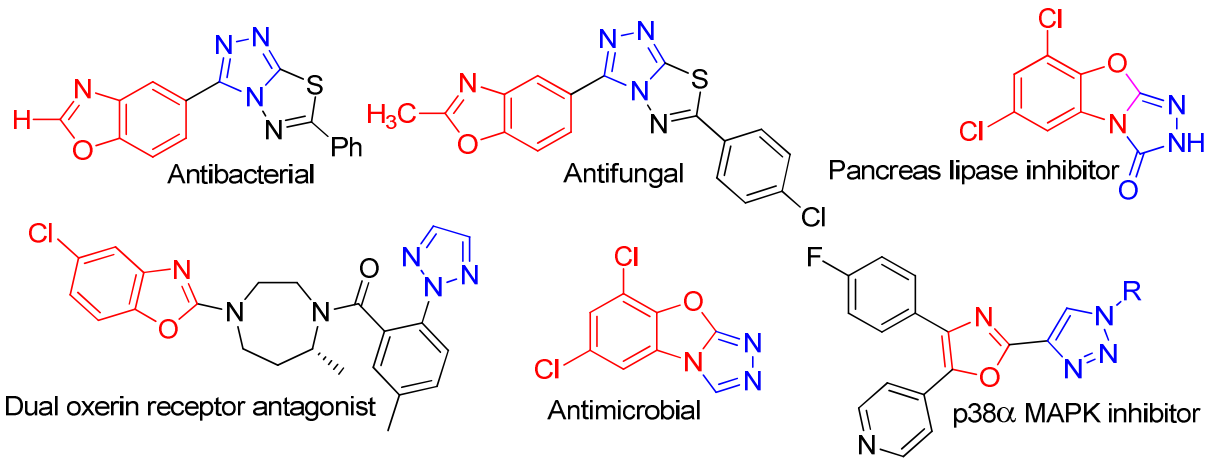


Figure 1. Benzoxazole-triazole scaffolds with varied biological activity [Ref.21-25].

Ubiquitous in organic molecules, unactivated carbon-hydrogen (C-H) bonds are among the simplest chemical moieties found in nature.[26] Although C-H bonds with dissociation energy in the range 90-100 Kcal/mol are typically inert to chemical transformations, directly utilizing them as reaction partners is highly desirable from the perspective of organic chemistry.[27] In target-oriented synthesis, methods to convert C-H bonds to new carbon-carbon (C-C) or carbon-heteroatom bonds can expedite the synthesis of architecturally complex intermediates en route to drugs, agrochemicals, or other biologically active molecules.[28] Similarly in diversity-oriented synthesis, an entire library of analogs can be synthesized directly from a lead compound via late stage C-H functionalization.[29] This is in contrast to the traditional methods that require one to return to the early stages to generate diversity which is costly in terms of chemical and energy inputs. Therefore, in view of time and atom economies, transition-metal catalyzed direct functionalization has gained enormous attention over the past decade as an effective and straightforward method. To this end, we have adopted a novel multi-component one-pot (MCOP) approach involving a copper catalyzed C-H activation of benzoxazole creating an alkynyl-heteroaryl linkage *in-situ*; followed by tandem cycloaddition with azides. To the best of our

knowledge, our method demonstrates the synthesis of an array of new benzoxazole linked triazole derivatives through direct C-H bond functionalization of sp^2-hybridized (hetero)aryl carbon with an sp-hybridized carbon of an alkyne coupled with 1,3-dipolar cycloaddition with azide in one pot. The biological efficacy of the synthesized molecules has been evaluated by carrying out antibacterial tests against the clinical isolates of *S. aureus* and *E. coli,* and *in vitro* cytotoxic activity against human cervical, breast, and liver cancer cell lines.

RESULTS AND DISCUSSION

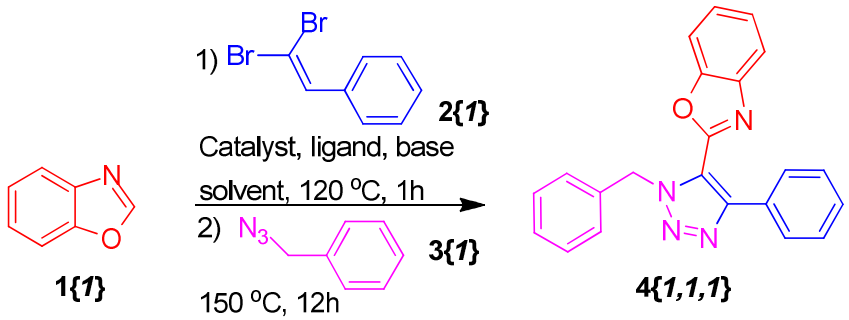
Synthesis of benzoxazole-triazole derivatives

The synthesis of benzoxazole linked triazoles was rationalized in two sequential steps in one pot. This involved an *in situ* generation of alkyne from the corresponding dibromo-olefin precursor *via* C-H activation of benzoxazole at C-2 position.[30,31] This was followed by 1,3-dipolar cycloaddition between the alkyne and benzyl azide to form the triazole ring. In the first step, benzoxazole **1{*1*}** (1.0 equiv.), (2,2-dibromovinyl)benzene **2{*1*}** (1.0 equiv.), CuI (20 mol%), PPh_3 (20 mol%) and LiO*t*-Bu (1.0 equiv.) in dioxane were heated at 120 °C for 1h. This was followed by addition of benzyl azide **3{*1*}** (1.0 equiv.) to the reaction mixture and heating the contents at 150 °C for another 12 h when the desired product 2-(3-Benzyl-5-phenyl-3H-[1,2,3]triazol-4-yl)-benzooxazole **4{*1,1,1*}** was formed. The product was isolated in 42% yield and was characterized by ^1H, ^{13}C NMR and mass spectrometry. The ^{1}HNMR spectrum of **4{*1,1,1*}** (**Figure S2**) showed 14 protons between 7-8 ppm corresponding to the protons of the three benzene rings. The benzylic protons appeared at 6.06 ppm which confirmed the presence of azide moiety in the product. Further, peaks at 134.98 and 141.01 ppm in ^{13}CNMR confirmed

formation of triazole ring. The HRMS (ESI) of the compound was found to be 375.1214, which is in accordance to the mass of **4{*1,1,1*}**.

Encouraged by these preliminary results, a detailed optimization study oriented towards understanding the influence of solvent, base, ligand, catalyst, time and temperature was taken up (**Table 1**). **1{*1*}** was chosen as the substrate for carrying out various optimization reactions. Screening of solvents such as DMF, acetonitrile, DMSO and dioxane indicated that dioxane was very specific for this reaction, as the reaction did not take place in all the other solvents tested (**Table 1**, **entry 1-4**). Next, evaluation of the reaction with inorganic bases weaker than LiO*t*-Bu such as KO*t*-Bu, NaH, KOH, K_2CO_3, Cs_2CO_3, and K_3PO_4 showed that except LiO*t*-Bu, none of the bases yielded any product in this reaction (**Table 1, entry 5-10**). Increasing the amount of LiO*t*-Bu to 2.0 and 3.0 equiv. increased the yield of **4{*1,1,1*}** to 49% and 64% respectively. Screening of copper and palladium salts as catalyst showed that product formation occurred only with CuI and CuBr as catalysts (**Table 1, entry 4, 12**) while no trace of product was seen with $Cu(OAc)_2$ and $Pd(OAc)_2$ (**Table 1, entry 13, 14**). To study the effect of the ligand in controlling the reaction yield, different ligands were used. It was found that in the absence of any ligand, no product was formed (**Table 1, entry 18**) and all the starting material was recovered. With nitrogen and oxygen containing ligands, however, poor yield of product **4{*1,1,1*}** was obtained (**Table 1, entry 15-17**). It was with triphenylphosphine that the yield was found to be maximal (**Table 1, entry 4**). After deriving the best conditions for the first step, optimization of temperature for the next step which is a thermal 1,3-dipolar cycloaddition reaction was carried out. It was found that cyclization proceeded only at or above 150 °C while at a lower temperature of 120 °C, no product was seen.

Table 1. Optimization of reaction conditions.[a]



Entry	Catalyst	Ligand	Base	Solvent	Yield[b] %
1.	CuI	PPh_3	LiO*t*-Bu	DMF	-[c]
2.	CuI	PPh_3	LiO*t*-Bu	CH_3CN	-[c]
3.	CuI	PPh_3	LiO*t*-Bu	DMSO	-[c]
4.	CuI	PPh_3	LiO*t*-Bu	Dioxane	64(61)[d](42)[e](49)[f]
5.	CuI	PPh_3	KO*t*-Bu	Dioxane	-[c]
6.	CuI	PPh_3	NaH	Dioxane	-[c]
7.	CuI	PPh_3	KOH	Dioxane	-[c]
8.	CuI	PPh_3	K_2CO_3	Dioxane	-[c]
9.	CuI	PPh_3	Cs_2CO_3	Dioxane	-[c]
10.	CuI	PPh_3	K_3PO_4	Dioxane	-[c]
11.	CuI	PPh_3	-	Dioxane	-[c]
12.	CuBr	PPh_3	LiO*t*-Bu	Dioxane	58
13.	$Cu(OAc)_2$	PPh_3	LiO*t*-Bu	Dioxane	-[c]
14.	$Pd(OAc)_2$	PPh_3	LiO*t*-Bu	Dioxane	-[c]
15.	CuI	L-Proline	LiO*t*-Bu	Dioxane	17
16.	CuI	2,2'-Bipyridine	LiO*t*-Bu	Dioxane	21
17.	CuI	8-Hydroxyquinoline	LiO*t*-Bu	Dioxane	14
18.	CuI	-	LiO*t*-Bu	Dioxane	-[c]

[a]Reaction conditions: **1{*1*}** (1.0 mmol), **2{*1*}** (1.0 mmol), **3{*1*}** (1.0 mmol), catalyst (10 mol%), ligand (15 mol%), base (3.0 equiv) in solvent (2 mL) for 1h at 120 ºC and for 12h at 150 ºC.

[b]Yield of isolated product, [c]reaction was run for 24h, [d]20 mol% CuI, [e]With 1.0 equiv. of LiO*t*-Bu, [f]With 2.0 equiv. of LiO*t*-Bu.

After optimization, the versatility of this protocol was ascertained by synthesizing a variety of benzoxazole-triazole scaffolds. Chemset of dibromoolefins **2** (**Figure 2**) with methoxy, methyl, bromo, nitro and trifluoromethyl substituents at *o/p* positions in the benzene ring was prepared in quantitative yields from the corresponding commercially available aldehydes *via* the Ramirez procedure.[32] Chemset of benzyl azides **3** (**Figure 2**) with various electron withdrawing substituents such as cyano, nitro, fluoro and difluoro was prepared quantitatively from the corresponding benzyl bromides by reaction with sodium azide. As shown in **Table 2**, the MCOP synthesis attempted thereafter with the synthesized building blocks was quite facile and enabled construction of a library of benzoxazole-triazole derivatives **4** in moderate to high yields.

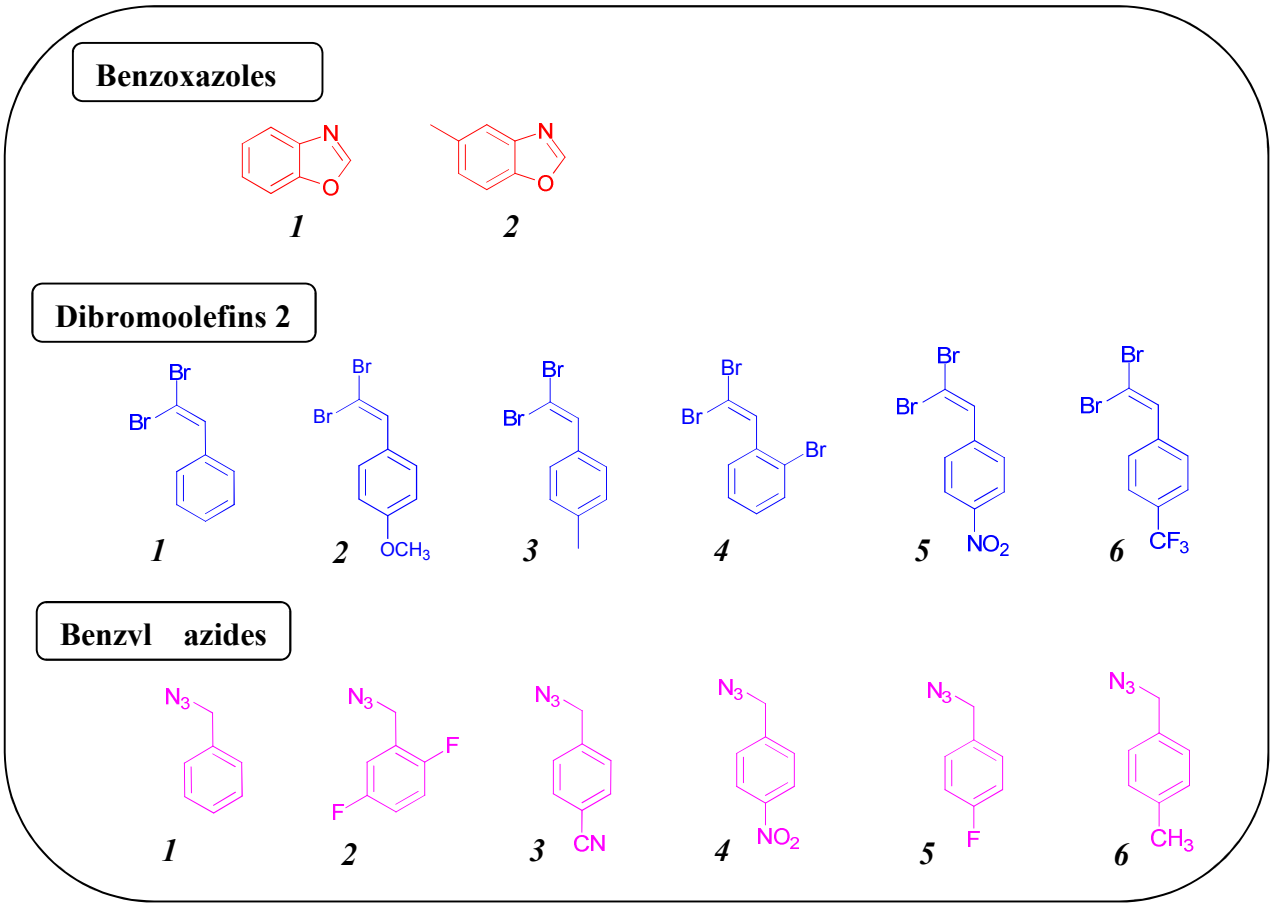
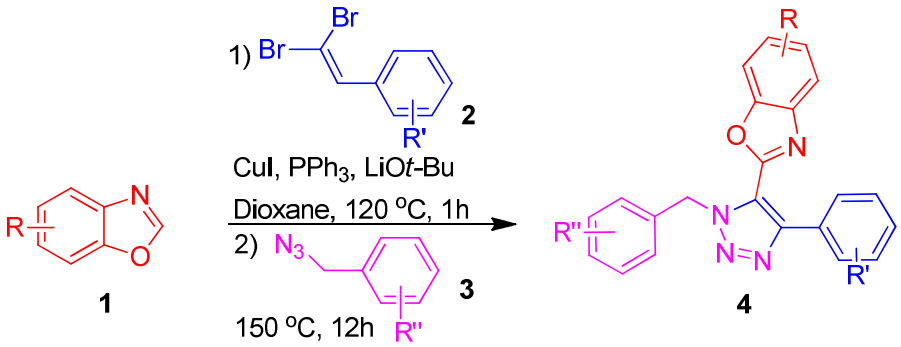


Figure 2. Structure and numbering of the building blocks.

It was found that of the chemset **2**, **2{*2*}** with *p*-methoxy substitution gave the highest yield of products with various azides (**Table 2, entry 5-9**). The reaction conditions were also well tolerated by *ortho*-bromo substituted olefin **2{*4*}** and 2-[3-Benzyl-5-(2-bromo-phenyl)-3H-[1,2,3]triazol-4-yl]-benzooxazole **4{*1,4,1*}** was obtained in moderate yield (**Table 2, entry 12**). However, the reaction failed completely with dibromoolefins having strongly deactivating groups like nitro **2{*5*}** and trifluoromethyl **2{*6*}** (**Table 2, entry 17, 18**). Screening of the chemset **3** showed that those with electron withdrawing substituents such as fluoro, cyano and nitro gave moderate to high yield of products in contrast to those with electron donating substituent like methyl **3{*6*}** which did not yield any product (**Table 2, entry 10**). By and large, the electronics on benzyl azide influenced the product yield and it followed the trend: cyano (**Table 2, entry 3, 7, 15**) > nitro (**Table 2, entry 4, 8, 16**) > difluoro (**Table 2, entry 2, 6, 14**) > fluoro (**Table 2, entry 9**). Moreover, it was found that the reaction with 5-methyl derivative of benzoxazole **1{*2*}** gave higher yield of products (**Table 2, entry 13-16**) compared to its unmethylated counterpart.

Interestingly, it was observed that of the two isomeric cycloaddition products possible, only one regioisomer of benzoxazole-triazole derivatives **4** was formed selectively. Since ^{1}HNMR and ^{13}CNMR spectra could not conclusively distinguish between the two isomers, the structural assignment of the regioisomeric triazoles was based on NOESY analysis.

Table 2. Substrate scope of reaction.[a]



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entry	product	yield[a] (%)
1	4{*1,1,1*}	64
2	4{*1,1,2*}	67
3	4{*1,1,3*}	72
4	4{*1,1,4*}	70
5	4{*1,2,1*}	69
6	4{*1,2,2*}	72
7	4{*1,2,3*}	75
8	4{*1,2,4*}	72
9	4{*1,2,5*}	62
10	4{*1,2,6*}	0
11	4{*1,3,2*}	60
12	4{*1,4,1*}	62
13	4{*2,2,1*}	73
14	4{*2,2,2*}	75
15	4{*2,2,3*}	81
16	4{*2,2,4*}	77

17	4{1,*5,1*}	0
18	4{1,*6,1*}	0

[a]Reaction conditions: **1** (1.0 mmol), dibromoolefin **2** (1.0 mmol), azide **3** (1.0 mmol), CuI (10 mol%), PPh_3 (15mol%), LiO*t*-Bu (3.0 equiv) in dioxane (2 mL) for 1h at 120 ºC and 12h at 150 ºC. [b]Yield of isolated product.

Figure 3 shows the NOESY ^{1}HNMR spectrum of the isomer **4{*1,2,1*}** formed from the reaction between **1{*1*}**, **2{*2*}** and **3{*1*}**. As is evident from the spectrum, cross-peaks are obtained due to interaction between benzyl protons (H_b) and *ortho*-aryl protons (H_c) of benzyl azide possible with both the isomers **4{*1,2,1*}** and **4{*1,2,1*}'**. However, absence of cross-peaks due to interaction between H_b and phenyl protons of the dibromoolefin fragment (H_a) suggested the regioisomer to be **4{*1,2,1*}** (see the encircled area in (**Figure 3**). If the product was **4{*1,2,1*}'**, then a cross peak should have appeared due to the spatial vicinity between H_a and H_b. The distance between H_a and H_b in **4{*1,2,1*}** is way too large to observe any cross peak. The reason behind the regioselective formation of single isomer can be rationalized based on the fact that benzoxazole acts as a powerful electron-withdrawing group thereby making the conjugated alkyne considerably electron-deficient and polarized.(33) This then directs the dipolar cycloaddition with azide in a specific controlled manner leading to a single regioisomer. Since chromatographic separation of regioisomeric triazoles is tedious, routes which can selectively yield a particular single isomer are attractive. In this regard, the approach offers a regiospecific transformation for synthesis of benzoxazole linked triazoles.

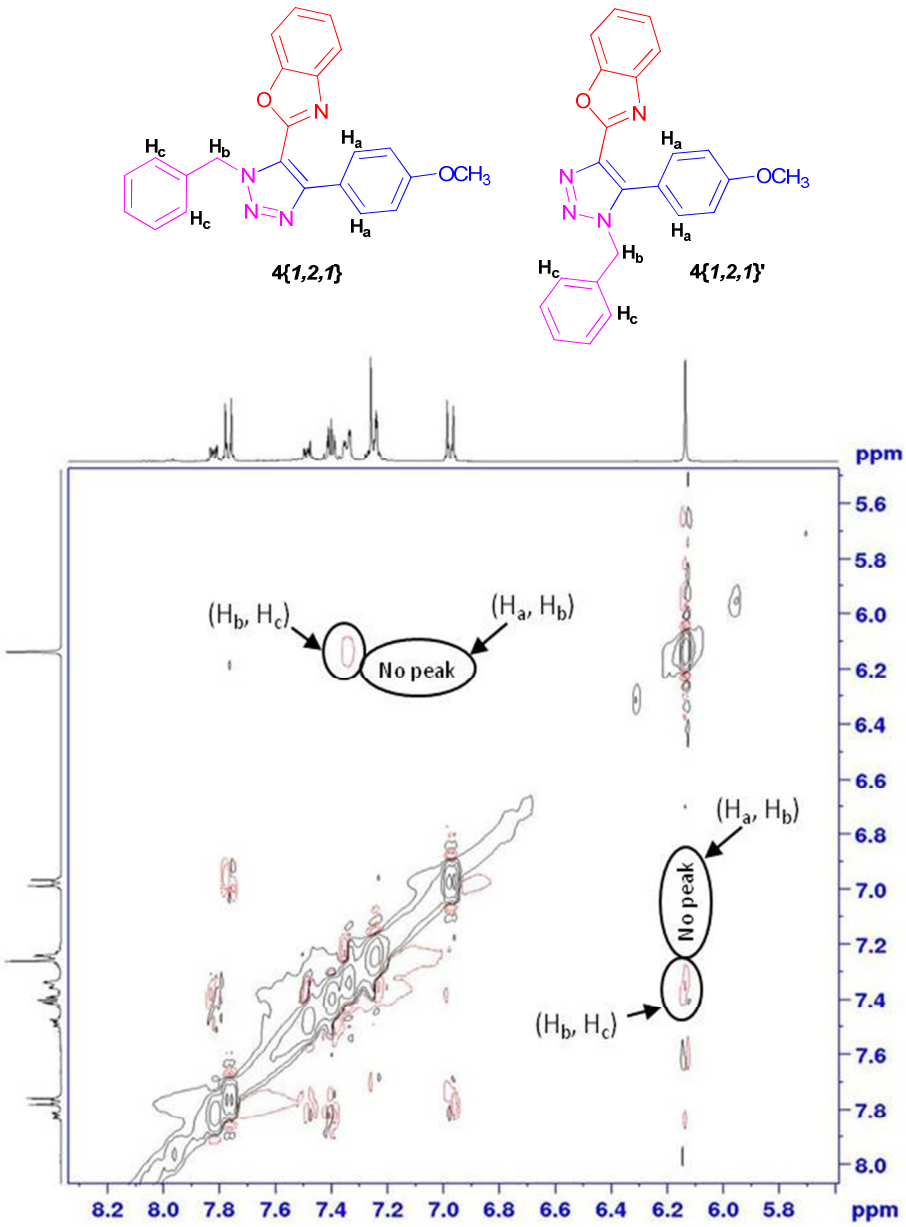

Figure 3. Two possible regioisomers (**4{*1,2,1*}** and **4{*1,2,1*}'**) and partial NOESY ^{1}HNMR spectrum.

Mechanism

An overview of the proposed mechanistic cycle with copper(I) as the active catalytic species **(A)** is shown in **figure 4**. The first step involves deprotonation of benzoxazole by LiO*t*-Bu followed

by lithium-copper trans-metallation to generate a three coordinated copper(I) intermediate **B**.[34] This is followed by generation of alkynyl bromide from 1,1-dibromoolefin by dehydrobromination effected by LiO*t*-Bu. The alkyne gets added onto **B** *via* oxidative addition resulting a four coordinated copper(III) complex **C**.[35-37] **D** is thereafter expelled from complex **C** *via* reductive elimination and the catalytic copper(I) species **A** is regenerated back. Formation of **D** was confirmed by isolating it at this stage and characterizing by ^{1}H and ^{13}C NMR spectroscopy. The next step is the tandem [3+2] cycloaddition of **D** with benzyl azide under thermal conditions to yield the benzoxazole-triazole derivative (**E**). The regioselectivity of **E** can be explained by considering the polarity of the triple bond conjugated with the benzoxazole moiety (partial positive charge is localized on β-carbon) and the dipole of the benzyl azide as depicted in **figure 4**. Due to this localization of charges, one regioisomer is selectively obtained.

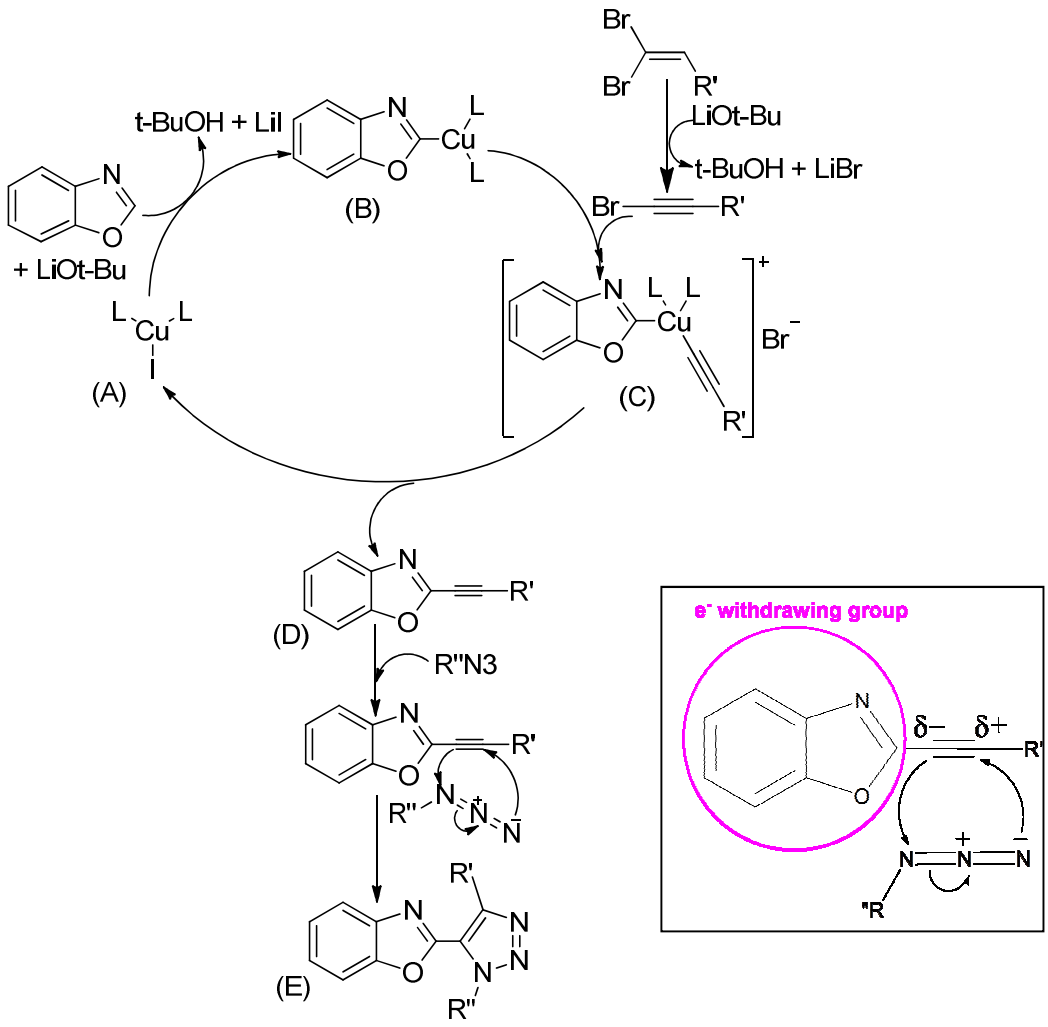

Figure 4. Proposed mechanism.

Antibacterial activity

The chemset **4** was screened for their *in vitro* antibacterial activity against the representative Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) clinical isolates and the results are summarized in **table 3**. A standard technique[38] based on the optical detection of the bacterial suspension at 620 nm (OD_{620}) was applied to evaluate the antibacterial activity of the test compounds. The antibacterial activity was evaluated by subtracting the OD for each

replicate at T0 from the OD for each replicate at T24. Cultures were incubated at 37 °C in an orbital shaker at 70 rpm to prevent adherence and clumping of cells. The antibacterial activity (*i.e.* growth inhibition) for the test sample was determined using the formula:

% Inhibition = [1- (OD test sample/OD of control)] x 100.

Table 3. Antibacterial activity of chemset **4** against *Staphylococcus aureus* and *Escherichia coli.*

Entry no.	Compound	*Staphylococcus aureus*		*Escherichia coli*	
		MIC ± SD (mg/mL)	IC_{50} ± SD (mg/mL)	MIC ± SD (mg/mL)	IC_{50} ± SD (mg/mL)
1.	4{*1,1,1*}	4.1 ± 0.16	8.2 ± 0.28	0.8 ± 0.03	2.1 ± 0.07
2.	4{*1,1,2*}	ND	ND	0.9 ± 0.04	2.7 ± 0.08
3.	4{*1,1,3*}	ND	ND	1.1 ± 0.05	3.2 ± 0.09
4.	4{*1,1,4*}	3.6 ± 0.14	5.2 ± 0.19	0.4 ± 0.03	1.8 ± 0.05
5.	4{*1,2,1*}	4.9 ± 0.20	6.1 ± 0.23	0.2 ± 0.01	1.2 ± 0.04
6.	4{*1,2,2*}	ND	ND	1.1 ± 0.05	2.1 ± 0.07
7.	4{*1,2,3*}	ND	ND	1.3 ± 0.06	3.1 ± 0.08
8.	4{*1,2,4*}	ND	ND	0.9 ± 0.02	2.2 ± 0.07
9.	4{*1,2,5*}	>11 ± 0.40	>15 ± 0.57	1.4 ± 0.06	2.7 ± 0.06
10.	4{*1,3,2*}	ND	ND	1.6 ± 0.06	2.2 ± 0.06
11.	4{*1,4,1*}	ND	ND	0.9 ± 0.03	2.4 ± 0.06
12.	4{*2,2,1*}	3.8 ± 0.15	>20 ± 0.76	1.3 ± 0.05	3.1 ± 0.07
13.	4{*2,2,2*}	>10 ± 0.39	>15 ± 0.51	1.4 ± 0.06	2.3 ± 0.06
14.	4{*2,2,3*}	ND	ND	1.2 ± 0.04	2.7 ± 0.08
15.	4{*2,2,4*}	> 19 ± 0.43	> 16 ± 0.58	0.9 ± 0.03	2.6 ± 0.07
16.	Ampicillin	0.42 ± 0.01	0.78 ± 0.03	ND	ND
17.	Gentamicin	ND	ND	0.09 ± 0.003	0.17 ± 0.006

Minimum Inhibitory Concentration (MIC): Exponentially growing bacterial cells were treated with different concentrations of test compounds for 24 h and cell growth inhibition was analyzed through the antimicrobial assay. IC_{50} is defined as the concentration at which a 50% decrease in cell number occurs compared to the control cultures in the absence of an inhibitor. The values represent the mean ± S.D. of three individual observations. Mean per cent decreases in cell number of three independent experiments were used to calculate the linear regression equation. ND = not determined

The antibacterial detection limits for each compound expressed as Minimum Inhibitory Concentration (MIC) and Inhibitory Concentration 50% (IC_{50}) were also determined. It was found that while most of the synthesized compounds showed little or no antibacterial activity, **4{*1,2,1*}** was reasonably effective against *E. coli* with the MIC and IC_{50} values of 0.2 ± 0.01 mg/mL and 1.2 ± 0.04 mg/mL respectively. This was followed by compound **4{*1,1,4*}** with MIC and IC_{50} values ranging between 0.4 ± 0.03 mg/mL and 1.8 ± 0.05 mg/mL respectively. The antibacterial activity against *S. aureus* in general, was less compared to *E. coli* isolates; possibly due to its resistance against the test compounds. To verify that the observed activity was only due to benzoxazole-triazole moiety, control experiments were performed in which *S. aureus* and *E. coli* cultures were treated with the carrier solution under same experimental conditions in the absence of test compounds. No antibacterial activity was seen in all these cases indicating that the effect was purely due to the test compounds in carrier solution. The antibacterial activity of **4{*1,2,1*}** with dose dependence was examined by evaluating the % inhibition against variable concentrations of **4{*1,2,1*}**. As can be seen in **Figure 5**, the % inhibition increased with increase in concentration from 0.2 mg/mL to 4.0 mg/mL. On further increasing the concentration, no significant increase in % inhibition was observed. Moreover, the inhibitory effect was more prominent against *E. coli* compared to *S. aureus.* A similar trend was also observed with compounds **4{*1,1,4*}** and **4{*1,1,1*}** against *E. coli* (data not shown). The % inhibition data followed the trend **4{*1,2,1*}>4{*1,1,4*}>4{*1,1,1*}** which is also in agreement with the results obtained for the IC_{50} values. The MIC and IC_{50} values for positive control of *S. aureus* was found to be 0.42 ± 0.01 mg/mL and 0.78 ± 0.03 mg/mL respectively (Table 3, entry 16). For the positive control of *E.coli*, the MIC and IC_{50} values were found to be 0.09 ± 0.003 mg/mL and 0.17 ± 0.006 mg/mL respectively (Table 3, entry 17).

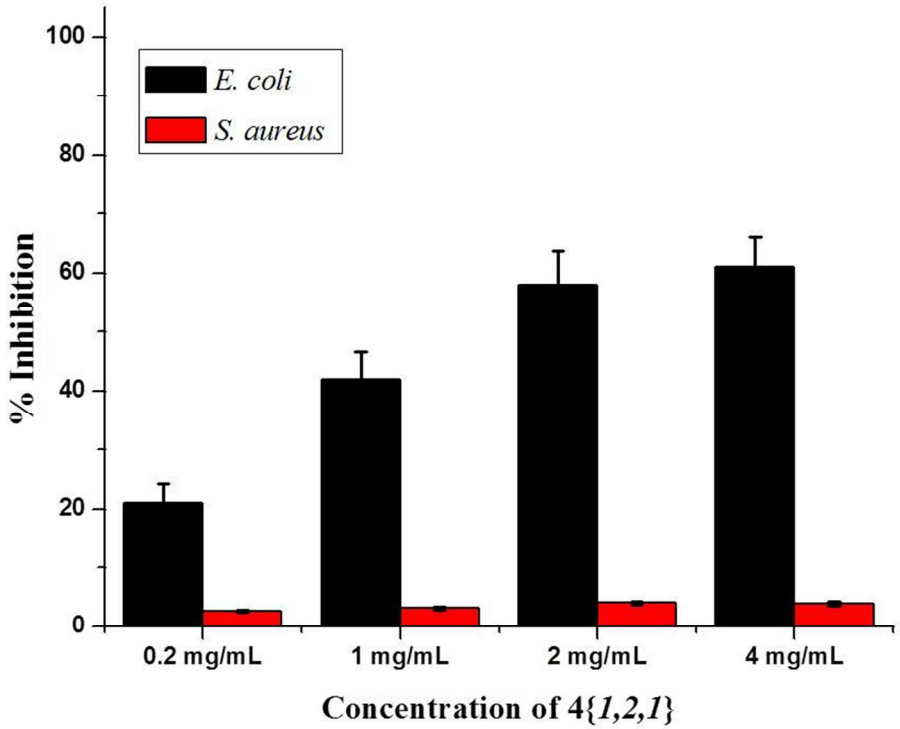

Figure 5. Histogram showing the dose dependent activity of **4{*1,2,1*}** towards *E. Coli* and *S. aureus*.

Cytotoxicity evaluation

The cytotoxicity of chemset **4** was assessed using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay which relies on the conversion of a tetrazolium compound (MTT) to purple colored formazan crystals by the activity of mitochondrial dehydrogenase present in cells. Cell viability was calculated using the equation: % Viability = [(OD compound treated cultures) / (OD untreated cell control cultures)] × 100. To begin with, the cytotoxicity of chemset **4** was determined at a concentration of 6.0 µg/mL against HeLa cells as shown in **figure 6**. For all the compounds except **4{*2,2,2*}**, we observed almost no cytotoxicity recovering greater than 80% of cells at all concentrations tested compared to untreated controls. Interestingly however, compound **4{*2,2,2*}** displayed moderate cytotoxicity against HeLa cells and was

compared with the positive control daunomycin (**figure 6**). While the % viability of cancer cells was 22 ± 0.86 % for daunomycin, for compound **4{*2,2,2*}** the % viability was 45 ± 1.84%. Encouraged by this result, we decided to carry out further cytotoxicity evaluation on other cell lines like SKBr3, and Hep G2 using **4{*2,2,2*}**.

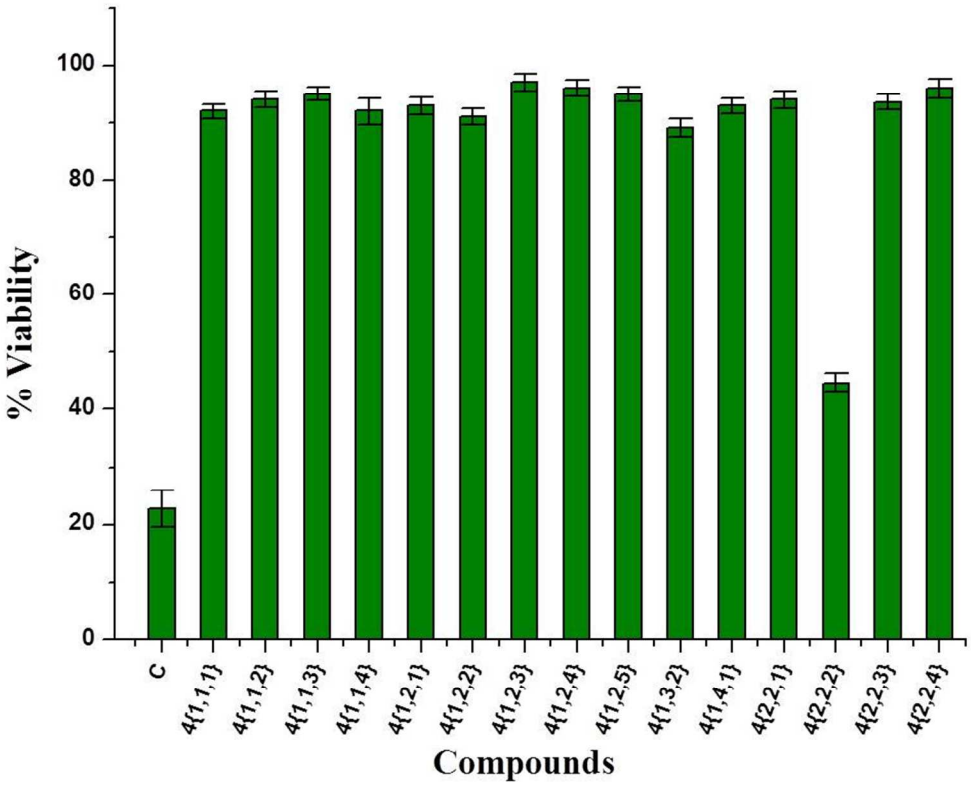

Figure 6. Cytotoxicity evaluation of chemset **4** on HeLa cell line using MTT assay at concentration 6.0 µg/mL. C represents the control experiment. The results shown are mean ± SD of five individual observations.

The IC_{50} values of compound **4{*2,2,2*}** were found to be 6.8 ± 0.34 µg/mL, 7.1 ± 0.41 µg/mL and 11.2 ± 0.64 µg/mL against HeLa, SKBr3 and Hep G2 cancer cells, respectively. A dose dependent cytotoxicity analysis of **4{*2,2,2*}** indicated that increasing the concentration of **4{*2,2,2*}** from 5 – 30 µg/mL led to decrease in the % viability of the three cancer cell lines as

shown in **figure 7A** indicating the potential applicability of this compound as a cancer therapeutic agent. These results suggested a potential anticancer activity vested in this new class of benzoxazole-triazole scaffolds.

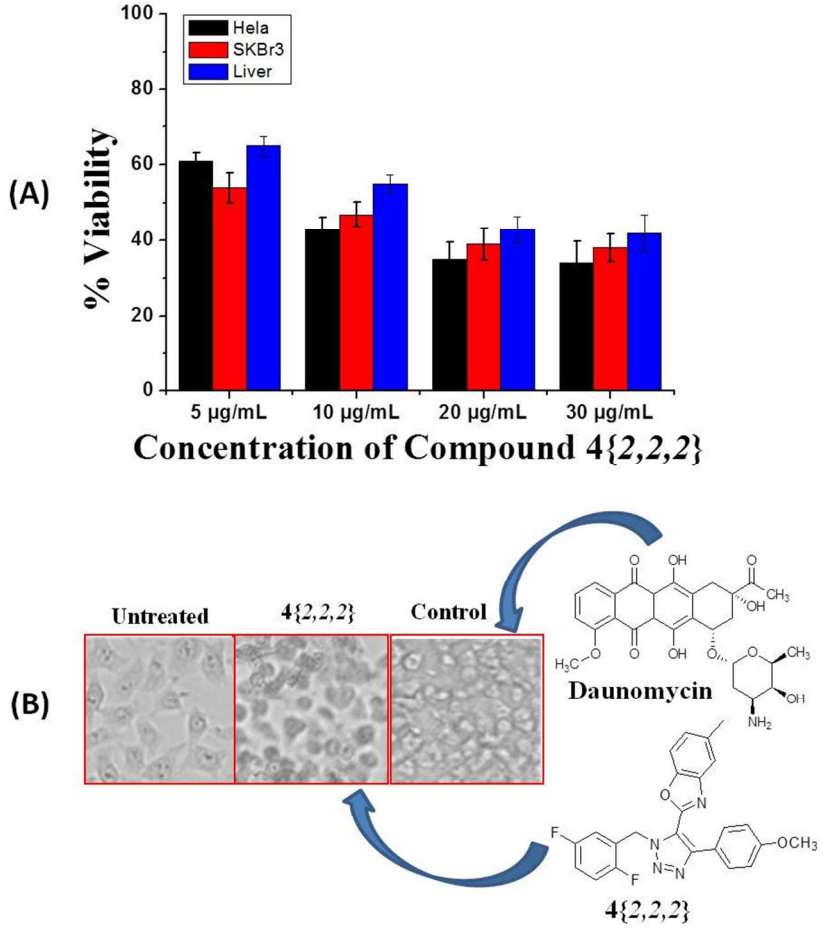

Figure 7. (A) Dose dependent cytotoxicity of **4{*2,2,2*}** on HeLa, SKBr3, Hep G2 cell lines using the MTT assay. The results shown are mean ± SD of five individual observations. (B) Light Micrographs of untreated and treated HeLa cancer cells. Left panel corresponds to untreated cells, middle panel represents the **4{*2,2,2*}** treated cells, and the right panel corresponds to daunomycin treated cells. Concentration of **4{*2,2,2*}** and daunomycin was 10 µg/mL. Cells were incubated at 37 °C in a 5% CO_2 humidified incubator.

To corroborate the observations of MTT assay, cells were also examined microscopically after 48 h of treatment with **4{*2,2,2*}**. It was found that the cells not only underwent a marked reduction in viability but also showed a change in their morphology as depicted in **figure 7B**. Cells appeared to be round and more granular which signifies some kind of apoptotic change in the cellular cytoplasm. As expected, the cell morphology with standard anticancer drug, daunomycin suffered a much greater disintegration and collapsed.

Conclusions

In conclusion, we report a regioselective synthesis and biological evaluation of a new class of benzoxazole-triazole derivatives. The compounds have been synthesized in moderate yields *via* facile and efficient multicomponent one pot synthetic approach. This has been realized by invoking a copper catalyzed direct C-H activation of benzoxazole followed by tandem thermal cycloadditon between alkyne and azide. The synthesized compounds have been evaluated for their antibacterial and anticancer activity. While most of the compounds were found to be weak antibacterials, **4{*1,1,1*}**, **4{*1,1,4*}**, and **4{*1,2,1*}** showed good gram-negative activity. Compound **4{*2,2,2*}** has been identified to have potent cytotoxic effect against cancer cell lines: HeLa, SKBr3, and Hep G2 and is comparable to the control daunomycin. Our work is the first report on the synthesis and application of this class of compounds as efficient anti-cancer agents. Future efforts shall be directed towards modifying the benzoxazole-triazole scaffold and developing promising lead structures through SAR as anticancer agents and establish their mechanism of action.

General experimental details:

Chemicals were either purchased or purified by standard techniques without special instructions. The products were purified by column chromatography on silica gel. ^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker DPX- 300 MHz spectrometer (^{1}H 300 MHz, ^{13}C 75 MHz) using $CDCl_3$ as the solvent with tetramethylsilane (TMS) as the internal standard at room temperature. All ^{13}C NMR spectra were obtained with complete proton decoupling. Chemical shifts are given in δ relative to TMS, the coupling constants J are given in Hz. The ESI-MS was performed on MICROTOF-II mass instrument. Data are reported as follows: chemical shift (in ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad), coupling constant (in Hz), and integration. Flash column chromatography was performed on silica gel (230–400 mesh) and analytical thin-layer chromatography was carried out using 250 μm commercial silica gel plates. Visualization of the developed chromatogram was performed by UV absorbance.

General procedure for the synthesis of 1,1-dibromoolefins 2:

1,1-dibromoolefins were synthesized using the Ramirez procedure starting from commercially available aldehydes. To a solution of benzaldehyde (10.0 mmol, 1.0 equiv.) and carbon tetrabromide (10.5 mmol, 1.5 equiv.) in dichloromethane (25 mL) at 0 °C, a solution of PPh_3 (21.0 mmol, 2.0 equiv.) in dichloromethane (10 mL) was added slowly. The reaction mixture was stirred until complete conversion of aldehyde was observed before being concentrated. The resulting crude was purified by flash chromatography over SiO_2 with pure hexane to afford compound **2{*1*}** as a colorless liquid (84% yield).

(2,2-dibromovinyl)benzene 2{*1*}:

^{1}H NMR ($CDCl_3$, 300MHz): δ 7.59 (d, J=6.3 Hz, 2H), 7.54 (s, 1H), 7.43 (d, J=6.6 Hz, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 136.90, 135.32, 128.61, 128.47, 89.75. The ^{1}H and ^{13}C NMR spectra of remaining members of chemset **2** are essentially similar to the reported data. [39-41]

General procedure for the synthesis of azides 3:

Following a reported procedure, a solution of sodium azide (1.5 equiv.) in water (8 mL) was added dropwise over 1 h to a solution of 2,5-difluorobenzyl bromide (1.0 equiv.) in acetone (20 mL) at 0°C. The reaction was allowed to warm up to 23 °C and stirred for 16 h. Acetone was removed under reduced pressure at 25 °C and the reaction mixture was extracted with hexane. Thereafter, the combined organic layers were put together and dried over Na_2SO_4 and the solvent was removed under reduced pressure to afford benzyl azide as colorless oil (100% yield).

2-azido-1,4-difluorobenzene 3{*2*}:

^{1}H NMR ($CDCl_3$, 300MHz): δ 6.97-6.87 (m, 3H), 4.26 (s, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 160.28, 158.27, 157.06, 155.05, 124.67, 116.83, 48.04. The ^{1}H and ^{13}C NMR spectra of remaining members of chemset **3** are essentially similar to the reported data. [42,43]

General procedure for one pot synthesis of benzoxazole-triazole derivatives 4:

A dried round bottom flask was charged with benzoxazole (0.840 mmol, 1.0 equiv.), CuI (0.084 mmol, 10 mol%), PPh_3 (0.126 mmol, 15 mol%), Li*O*t*Bu (2.521 mmol, 3.0 equiv.). 2,2-dibromovinylbenzene (0.840 mmol, 1.0 equiv.) was diluted into dioxane (10 mL) and the solution was added to the R.B. flask. The reaction mixture was then refluxed with water condenser in a pre-heated oil bath at 120 °C for 1h. After this 2,5-difluorobenzyl azide (1.0 equiv.) was added and the contents were heated up to 150 ^{o}C and stirred for 12 h.The reaction mixture was diluted with EtOAc and water was added. This mixture was extracted with EtOAc and the combined organic layers were put together and dried upon Na_2SO_4. Solvents were

removed under reduced pressure and the crude was purified by column chromatography on silica gel to afford **4{*1,1,2*}** as a white solid.

2-[3-(2,5-Difluoro-benzyl)-5-phenyl-3H-[1,2,3]triazol-4-yl]-benzooxazole 4{*1,1,2*}:

^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.60-7.58 (m, 3H), 7.31-7.27 (m, 3H), 7.26 (d, J=1.8 Hz, 1H), 7.23 (d, J=4.2 Hz, 1H), 7.21 (d, J=2.7 Hz, 1H), 7.06 (s, 1H), 6.85-6.64 (m, 3H), 5.98 (s, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 160.29, 157.89, 157.07, 154.69, 152.73, 150.01, 149.01, 140.94, 129.73, 129.22, 128.91, 128.42, 126.46, 125.30, 121.93, 120.67, 116.10, 115.82, 110.96, 47.48; HRMS (ESI): calcd for $C_{24}H_{14}F_2N_4O$ $[M+H]^+$ 389.1208, found 389.1215.

General procedure for *in vitro* antibacterial activity:

The bacterial cultures of *S aureus* and *E coli* were isolated from the clinical samples and characterized using routine laboratory technique. *S aureus* and *E coli* were grown for 24 h on blood agar plates (5% sheep red blood cells, and tryptic soy agar (TSA)) and macConkey agar at 37 °C, respectively. The cells were suspended in 1 % saline solution and the optical density of 0.6 at 600 nm (OD_{600}) was adjusted to obtain a density of 7 x 10^5 colony forming units per mL (CFU/mL), which was confirmed by standard plate count method. The antibacterial test was performed using an optical method. At first, the stock solution was prepared in DMSO and further diluted using the sterile PBS. The sample was always freshly prepared and thoroughly mixed before performing the antibacterial test. The desired concentrations of the samples were treated with the bacterial cultures aseptically and the samples were incubated for 24 h at 37 °C in shaker incubator. After incubation, the antibacterial activity was calculated by recording the OD_{600} and the percentage bacterial growth inhibition was calculated as formula given below.

$$\% \text{ Inhibition} = [1 - (\text{OD test sample}/\text{OD of control})] \times 100.$$

Each of the experiments was performed in triplicate. Control experiments were also performed without the test samples.

General procedure for *in vitro* anticancer activity:

The cytotoxicity/anticancer activity of the compounds was assessed using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay which relies on the conversion of a tetrazolium compound (MTT) to purple colored formazan crystals by the activity of mitochondrial dehydrogenase present in cells. Cell viability was calculated using the equation: % Viability = [(OD compound treated cultures) / (OD untreated cell control cultures)] × 100. The analysis method was as follows. Briefly, cell lines were cultured in T-75 tissue culture flasks (Nunc, Denmark) at 37 °C in a 5% CO_2 humidified incubator in suitable media and supplements. Cells were seeded in separate wells containing 100 μL medium at a final density of 2×10^4 cells/well, in a 96 well microtiter plates under the similar experimental conditions. After overnight incubation, the cells were treated with various concentrations of the test compounds (5–50 μg/mL) or dimethyl sulfoxide (DMSO) (carrier solvent) separately in a final volume of 200 μL in triplicates. After 24 h of incubation, 10 μL of MTT reagent (5 mg/mL) was added to each well and the plate was incubated at 37 °C in the dark for 4 h. After this, the media along with MTT was removed and the formazan crystals were solubilised in DMSO (100 μL/well). The reduction of MTT was measured by recording the absorbance at 570 nm by using a microplate reader. Effects of the test compounds on cell viability were calculated using cells treated with DMSO as control.

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SUPPORTING INFORMATION

^{1}HNMR, ^{13}CNMR, and mass spectra of chemset **4.** This material is available free of charge via the Internet at http://pubs.acs.org.

ABBREVIATIONS: CuI (copper(I) iodide), DMSO (dimethylsulfoxide), DMF (*N,N*-dimethylformamide), NOESY (Nuclear Overhauser effect spectroscopy).

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Graphic Table of Contents