

One-Pot Sequential Alkynylation and Cycloaddition: Regioselective Construction and Biological Evaluation of Novel Benzoxazole–Triazole Derivatives

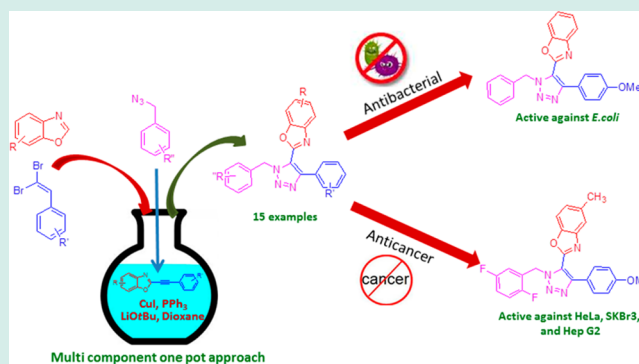
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S Supporting Information

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, multicomponent, 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 combination of 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.

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



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-HIV²⁰ 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.

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 multicomponent one-pot (MCOP) approach involving a copper catalyzed C–H activation of benzoxazole creating an alkyne–heteroaryl linkage in situ; followed by tandem

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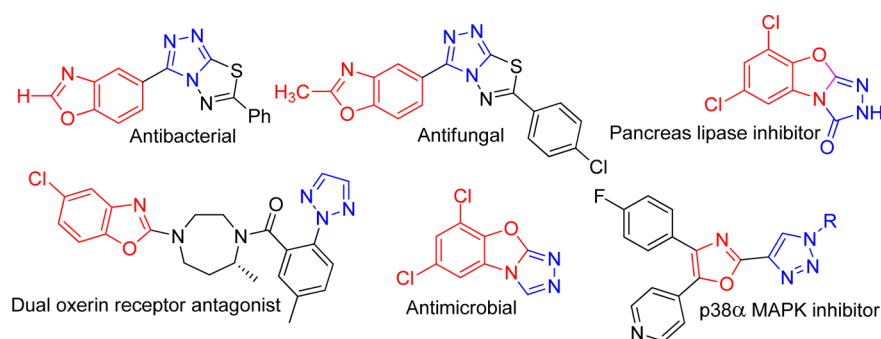
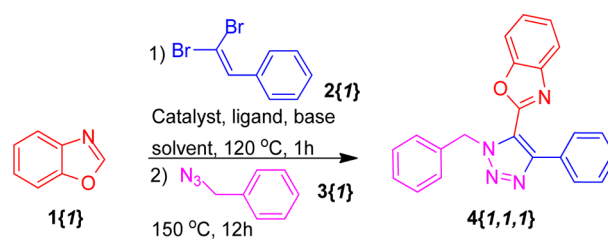


Figure 1. Benzoxazole-triazole scaffolds with varied biological activity.^{21–25}

Table 1. Optimization of Reaction Conditions^a



entry	catalyst	ligand	base	solvent	yield ^b (%)
1	CuI	PPh ₃	LiOt-Bu	DMF	— ^c
2	CuI	PPh ₃	LiOt-Bu	CH ₃ CN	— ^c
3	CuI	PPh ₃	LiOt-Bu	DMSO	— ^c
4	CuI	PPh ₃	LiOt-Bu	dioxane	64(61), ^d (42), ^e (49) ^f
5	CuI	PPh ₃	KOt-Bu	dioxane	— ^c
6	CuI	PPh ₃	NaH	dioxane	— ^c
7	CuI	PPh ₃	KOH	dioxane	— ^c
8	CuI	PPh ₃	K ₂ CO ₃	dioxane	— ^c
9	CuI	PPh ₃	Cs ₂ CO ₃	dioxane	— ^c
10	CuI	PPh ₃	K ₃ PO ₄	dioxane	— ^c
11	CuI	PPh ₃	—	dioxane	— ^c
12	CuBr	PPh ₃	LiOt-Bu	dioxane	58
13	Cu(OAc) ₂	PPh ₃	LiOt-Bu	dioxane	— ^c
14	Pd(OAc) ₂	PPh ₃	LiOt-Bu	dioxane	— ^c
15	CuI	L-proline	LiOt-Bu	dioxane	17
16	CuI	2,2'-bipyridine	LiOt-Bu	dioxane	21
17	CuI	8-hydroxyquinoline	LiOt-Bu	dioxane	14
18	CuI	—	LiOt-Bu	dioxane	— ^c

^aReaction 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 °C and for 12h at 150 °C. ^bYield of isolated product. ^cReaction was run for 24 h. ^d20 mol % CuI. ^eWith 1.0 equiv of LiOt-Bu. ^fWith 2.0 equiv of LiOt-Bu.

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 sp²-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 *Staphylococcus aureus* and *Escherichia 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 %), PPh₃ (20 mol %), and LiOt-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)-benzoxazole 4{1,1,1} was formed. The product was isolated in 42% yield and was characterized by ¹H, ¹³C NMR and mass spectrometry. The ¹H NMR spectrum of 4{1,1,1} (Supporting Information Figure S2) showed 14 protons between 7 and 8 ppm corresponding to the protons of the three benzene rings. The benzylic protons appeared at 6.06

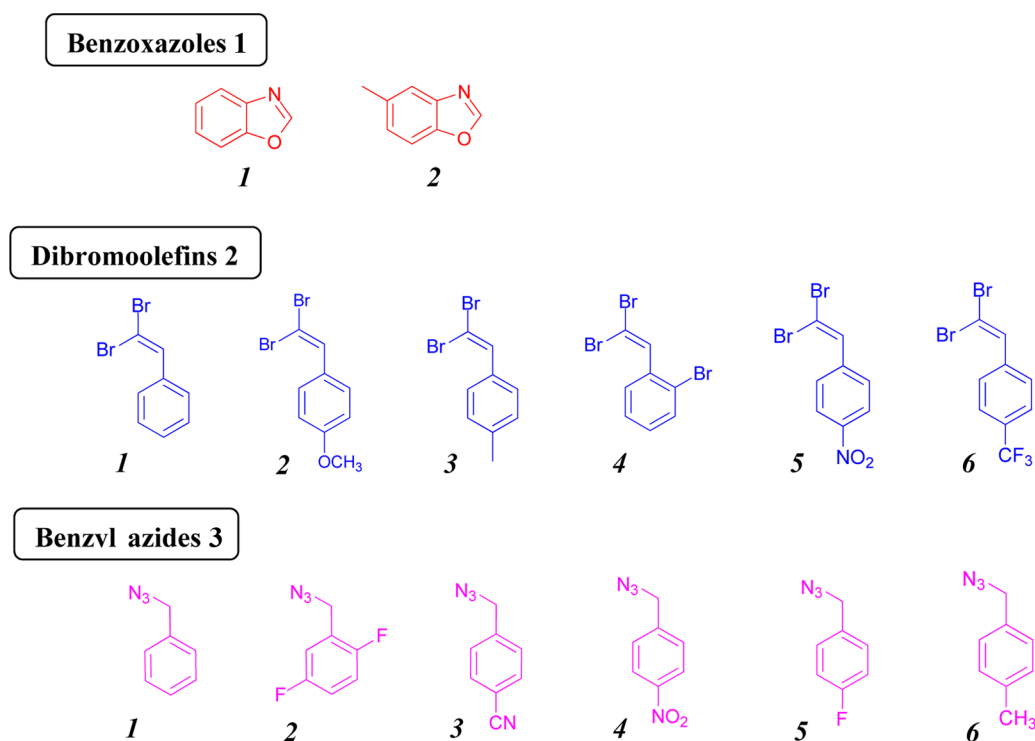


Figure 2. Structure and numbering of the building blocks.

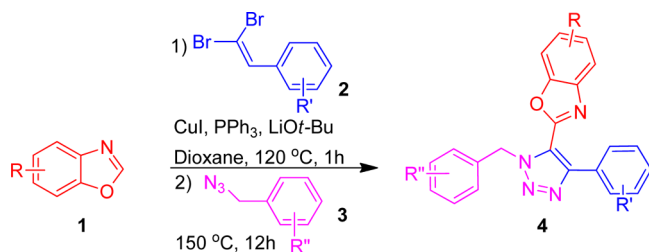
ppm which confirmed the presence of azide moiety in the product. Further, peaks at 134.98 and 141.01 ppm in ^{13}C NMR confirmed formation of triazole ring. The HRMS (ESI) of the compound was found to be 375.1214, which is in accordance to the mass of 4{1,1,1}.

Encouraged by these preliminary results, a detailed optimization study oriented toward 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, entries 1–4). Next, evaluation of the reaction with inorganic bases weaker than LiOt-Bu , such as KOt-Bu , NaH , KOH , K_2CO_3 , Cs_2CO_3 , and K_3PO_4 , showed that except LiOt-Bu , none of the bases yielded any product in this reaction (Table 1, entries 5–10). Increasing the amount of LiOt-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, entries 4, 12), while no trace of product was seen with $\text{Cu}(\text{OAc})_2$ and $\text{Pd}(\text{OAc})_2$ (Table 1, entries 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, entries 15–17). It was with triphenylphosphine that the yield was found to be maximal (Table 1, entry 4). After derivation of 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.

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.

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, entries 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]-benzoxazole 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, entries 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, entries 3, 7, 15) > nitro (Table 2, entries 4, 8, 16) > difluoro (Table 2, entries 2, 6, 14) > fluoro (Table 2, entry 9). Moreover, it was found that the

Table 2. Substrate Scope of Reaction^a

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

^aReaction conditions: **1** (1.0 mmol), dibromoolefin **2** (1.0 mmol), azide **3** (1.0 mmol), CuI (10 mol %), PPh₃ (15 mol %), LiOt-Bu (3.0 equiv) in dioxane (2 mL) for 1 h at 120 °C and 12 h at 150 °C. ^bYield of isolated product.

reaction with 5-methyl derivative of benzoxazole **1**{2} gave higher yield of products (Table 2, entries 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 ¹H NMR and ¹³C NMR spectra could not conclusively distinguish between the two isomers, the structural assignment of the regioisomeric triazoles was based on NOESY analysis.

Figure 3 shows the NOESY ¹H NMR 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 because of 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 regioselective transformation for synthesis of benzoxazole linked triazoles.

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 LiOt-Bu followed by lithium–copper transmetalation 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 LiOt-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 ¹H and ¹³C 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. Because of this localization of charges, one regioisomer is selectively obtained.

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 technique³⁸ based on the optical detection of the bacterial suspension at 620 nm (OD₆₂₀) 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:

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

The antibacterial detection limits for each compound expressed as minimum inhibitory concentration (MIC) and inhibitory concentration 50% (IC₅₀) 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 IC₅₀ values of 0.2 ± 0.01 and 1.2 ± 0.04 mg/mL, respectively. This was followed by compound 4{1,1,4} with MIC and IC₅₀ values ranging between 0.4 ± 0.03 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 percent inhibition against variable concentrations of 4{1,2,1}. As can be seen in

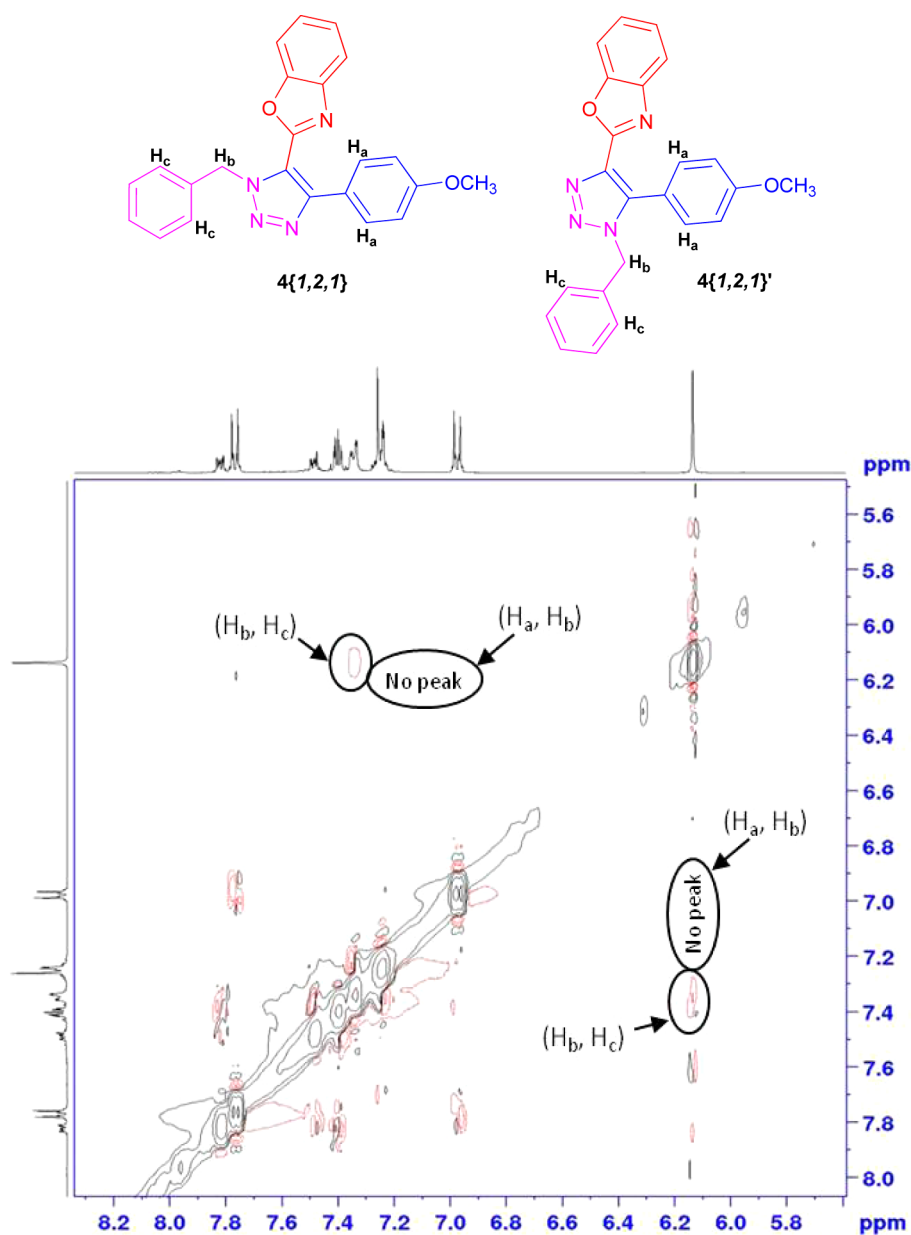


Figure 3. Two possible regioisomers ($4\{1,2,1\}$ and $4\{1,2,1'\}$) and partial NOESY ^1H NMR spectrum.

Figure 5, the percent inhibition increased with increase in concentration from 0.2 to 4.0 mg/mL. On further increasing the concentration, no significant increase in percent 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 percent 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 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 and 0.17 ± 0.006 mg/mL, respectively (Table 3, entry 17).

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 = $[(\text{OD compound treated cultures})/(\text{OD untreated cell control cultures})] \times 100$. To begin with, the cytotoxicity of chemset 4 was determined at a concentration of $6.0 \mu\text{g/mL}$ 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 $22 \pm 0.86\%$ for daunomycin, for compound $4\{2,2,2\}$ the % viability was $45 \pm 1.84\%$. Encouraged by this result, we decided to carry out further cytotoxicity evaluation on other cell lines like SKBr3, and Hep G2 using $4\{2,2,2\}$.

The IC_{50} values of compound $4\{2,2,2\}$ were found to be 6.8 ± 0.34 , 7.1 ± 0.41 , and $11.2 \pm 0.64 \mu\text{g/mL}$ against HeLa,

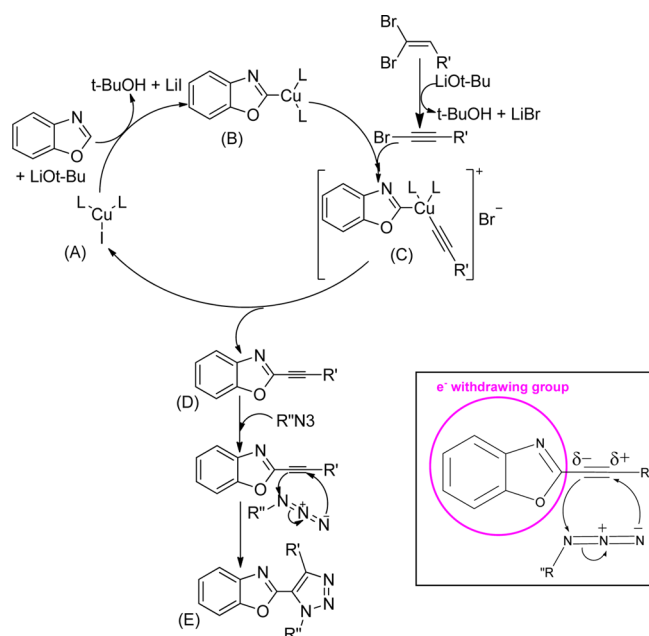


Figure 4. Proposed mechanism.

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 $\mu\text{g/mL}$ led to decrease in the percent 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.

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

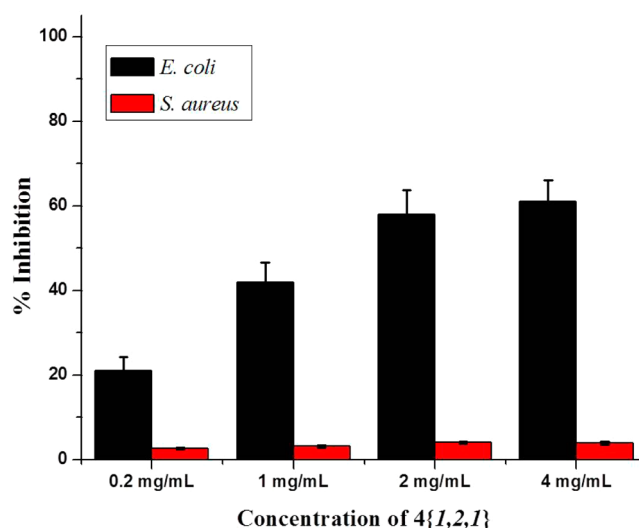


Figure 5. Histogram showing the dose dependent activity of 4{1,2,1} toward *E. coli* and *S. aureus*.

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

Table 3. Antibacterial Activity of Chemset 4 against *Staphylococcus aureus* and *Escherichia coli*^a

entry	compound	<i>Staphylococcus aureus</i>		<i>Escherichia coli</i>	
		MIC \pm SD (mg/mL)	IC ₅₀ \pm SD (mg/mL)	MIC \pm SD (mg/mL)	IC ₅₀ \pm SD (mg/mL)
1	4{1,1,1}	4.1 \pm 0.16	8.2 \pm 0.28	0.8 \pm 0.03	2.1 \pm 0.07
2	4{1,1,2}	ND	ND	0.9 \pm 0.04	2.7 \pm 0.08
3	4{1,1,3}	ND	ND	1.1 \pm 0.05	3.2 \pm 0.09
4	4{1,1,4}	3.6 \pm 0.14	5.2 \pm 0.19	0.4 \pm 0.03	1.8 \pm 0.05
5	4{1,2,1}	4.9 \pm 0.20	6.1 \pm 0.23	0.2 \pm 0.01	1.2 \pm 0.04
6	4{1,2,2}	ND	ND	1.1 \pm 0.05	2.1 \pm 0.07
7	4{1,2,3}	ND	ND	1.3 \pm 0.06	3.1 \pm 0.08
8	4{1,2,4}	ND	ND	0.9 \pm 0.02	2.2 \pm 0.07
9	4{1,2,5}	>11 \pm 0.40	>15 \pm 0.57	1.4 \pm 0.06	2.7 \pm 0.06
10	4{1,3,2}	ND	ND	1.6 \pm 0.06	2.2 \pm 0.06
11	4{1,4,1}	ND	ND	0.9 \pm 0.03	2.4 \pm 0.06
12	4{2,2,1}	3.8 \pm 0.15	>20 \pm 0.76	1.3 \pm 0.05	3.1 \pm 0.07
13	4{2,2,2}	>10 \pm 0.39	>15 \pm 0.51	1.4 \pm 0.06	2.3 \pm 0.06
14	4{2,2,3}	ND	ND	1.2 \pm 0.04	2.7 \pm 0.08
15	4{2,2,4}	>19 \pm 0.43	>16 \pm 0.58	0.9 \pm 0.03	2.6 \pm 0.07
16	ampicillin	0.42 \pm 0.01	0.78 \pm 0.03	ND	ND
17	gentamicin	ND	ND	0.09 \pm 0.003	0.17 \pm 0.006

^aMinimum 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. IC₅₀ 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 \pm SD of three individual observations. Mean percent decreases in cell number of three independent experiments were used to calculate the linear regression equation. ND = not determined

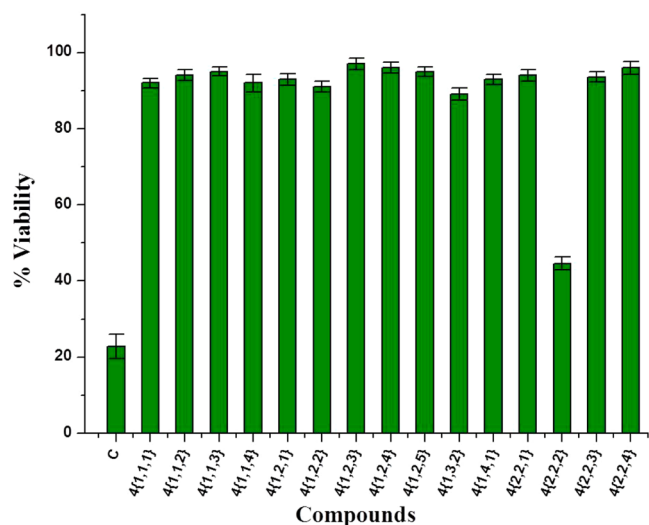


Figure 6. Cytotoxicity evaluation of chemset 4 on HeLa cell line using MTT assay at concentration 6.0 $\mu\text{g/mL}$. C represents the control experiment. The results shown are mean \pm SD of five individual observations.

thermal cycloaddition 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 anticancer agents. Future efforts shall be directed toward modifying the benzoxazole-triazole scaffold and developing promising lead structures through SAR as anticancer agents and establish their mechanism of action.

EXPERIMENTAL PROCEDURES

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. ^1H NMR and ^{13}C NMR spectra were measured on a Bruker DPX-300 MHz spectrometer (^1H 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),

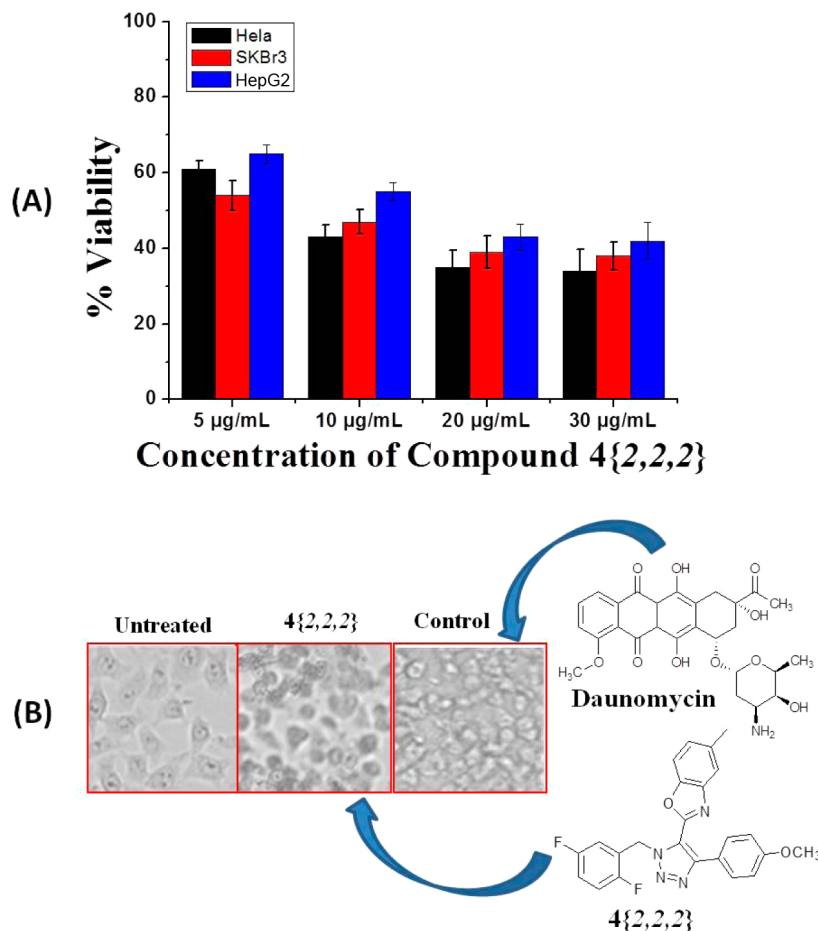


Figure 7. (A) Dose-dependent cytotoxicity of 4{2,2,2} on HeLa, SKBr3, Hep G2 cell lines using the MTT assay. The results shown are mean \pm SD of five individual observations. (B) Light Micrographs of untreated and treated HeLa cancer cells. Left panel corresponds to untreated cells, middle panel represents 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 $\mu\text{g/mL}$. Cells were incubated at 37 $^\circ\text{C}$ in a 5% CO_2 humidified incubator.

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}. ^1H NMR (CDCl_3 , 300 MHz): 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 ^1H 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}. ^1H NMR (CDCl_3 , 300 MHz): 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 ^1H 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 %), and LiOtBu (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 preheated oil bath at 120 °C for 1 h. After this, 2,5-difluorobenzyl azide (1.0 equiv) was added, and the contents were heated up to 150 °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]-benzoxazole 4{1,1,2}. ^1H 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 $\text{C}_{24}\text{H}_{14}\text{F}_2\text{N}_4\text{O}$ [$\text{M} + \text{H}$]⁺ 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 7×10^5 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)] \times 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 $\mu\text{g}/\text{mL}$) 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 solubilized in DMSO (100 $\mu\text{L}/\text{well}$). 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.

■ ASSOCIATED CONTENT

Supporting Information

^1H NMR, ^{13}C NMR, and mass spectra of chemset 4. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

CuI, copper(I) iodide; DMSO, dimethyl sulfoxide; DMF, *N,N*-dimethylformamide; NOESY, nuclear overhauser effect spectroscopy

REFERENCES

- (1) Whiting, M.; Tripp, J. C.; Lin, Y. C.; Lindstrom, W.; Olson, A. J.; Elder, J. H.; Sharpless, K. B.; Fokin, V. V. Discovery and structure-activity profiling of novel inhibitors of human immunodeficiency virus type 1 protease enabled by the copper(I)-catalyzed synthesis of 1,2,3-triazoles and their further functionalization. *J. Med. Chem.* **2006**, *49*, 7697–7710.
- (2) Alvarez, R.; Velázquez, S.; Felix, A. S.; Aquaro, S.; Clercq, E. D.; Perno, C. F.; Karlsson, A.; Balzarini, J.; Camarasa, M. J. 1,2,3-Triazole-[2',5'-Bis-*O*-(tertbutyl dimethylsilyl)- β -D-ribofuranosyl]-3'-spiro-5"- (4"-amino-1",2"-oxathiole 2",2"-dioxide) (TSAO) analogues: Synthesis and anti-HIV-1 activity. *J. Med. Chem.* **1994**, *37*, 4185–4194.
- (3) Fu, T.-h.; Li, Y.; Thaker, H. D.; Scott, R. W.; Tew, G. N. Expedient synthesis of SMAMPs via click chemistry. *ACS Med. Chem. Lett.* **2013**, *4*, 841–845.
- (4) Bel'Skaya, N. P.; Demina, M. A.; Sapognikova, S. G.; Fan, Z.-J.; Zhang, H.-K.; Dehaen, W.; Bakulev, V. A. Synthesis and oxidative cyclization of 2-arylhydrazono-2-cyanoacetamides to 5-amino-2-aryl-2H-[1,2,3]triazole-4-carbonitrile. *Arkivoc* **2008**, *16*, 9–21.
- (5) Genin, M. J.; Allwine, D. A.; Anderson, D. J.; Barbachyn, M. R.; Emmert, D. E.; Garmon, S. A.; Graber, D. R.; Grega, K. C.; Hester, J. B.; Hutchinson, D. K.; Morris, J.; Reischer, R. J.; Ford, C. W.; Zurenko, G. E.; Hamel, J. C.; Schaadt, R. D.; Stapert, D.; Yagi, B. H. Substituent effects on the antibacterial activity of nitrogen-carbon-linked (azolyphenyl)oxazolidinones with expanded activity against the fastidious gram-negative organisms *Haemophilus influenzae* and *Moraxella catarrhalis*. *J. Med. Chem.* **2000**, *43*, 953–970.
- (6) Reck, F.; Zhou, F.; Girardot, M.; Kern, G.; Eyermann, C. J.; Hales, N. J.; Ramsay, R. R.; Gravestock, M. B. Identification of 4-substituted 1,2,3-triazoles as novel oxazolidinone antibacterial agents with reduced activity against monoamine oxidase A. *J. Med. Chem.* **2005**, *48*, 499–506.
- (7) Beena; Kumar, N.; Rohilla, R. K.; Roy, N.; Rawat, D. S. Synthesis and antibacterial activity evaluation of metronidazole–triazole conjugates. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1396–1398.
- (8) Bakunov, S. A.; Bakunova, S. M.; Wenzler, T.; Ghebru, M.; Werbovetz, K. A.; Brun, R.; Tidwell, R. R. Synthesis and antiprotozoal activity of cationic 1,4-diphenyl-1H-1,2,3-triazoles. *J. Med. Chem.* **2010**, *53*, 254–272.
- (9) Shafi, S.; Alam, M. M.; Mulakayala, N.; Mulakayala, C.; Vanaja, G.; Kalle, A. M.; Pallud, R.; Alam, M. S. Synthesis of novel 2-mercapto benzothiazole and 1,2,3-triazole based bis-heterocycles: Their anti-inflammatory and anti-nociceptive activities. *Eur. J. Med. Chem.* **2012**, *49*, 324–333.
- (10) Elamari, H.; Slimi, R.; Chabot, G. G.; Quentin, L.; Scherman, D.; Girard, C. Synthesis and in vitro evaluation of potential anticancer activity of bis-alkyne and their mono- and bis-1,2,3-triazole derivatives. *Eur. J. Med. Chem.* **2013**, *60*, 360–364.
- (11) Li, W.-T.; Wu, W.-H.; Tang, C.-H.; Tai, R.; Chen, S.-T. One-pot tandem copper-catalyzed library synthesis of 1-thiazolyl-1,2,3-triazoles as anticancer agents. *ACS Comb. Sci.* **2011**, *13*, 72–78.
- (12) Patil, V.; Guarrant, W.; Chen, P. C.; Gryder, B.; Benicewicz, D. B.; Khan, S. I.; Tekwani, B. L.; Oyelere, A. K. Antimalarial and antileishmanial activities of histone deacetylase inhibitors with triazole-linked cap group. *Bioorg. Med. Chem.* **2010**, *18*, 415–425.
- (13) Diana, G. D.; Nitz, T. J. 1,2,4-Oxadiazolyl-phenoxyalkylisoxazoles and their use as antiviral agents. Eur. Patent EP 566199, 1993.
- (14) Oksuzoglu, E.; Gulbas, B. T.; Alper, S.; Arpacı, O. T.; Ertan, T.; Yildiz, I.; Diri, N.; Aki, E. S.; Yalcin, I. Some benzoxazoles and benzimidazoles as DNA topoisomerase I and II inhibitors. *J. Enzyme Inhib. Med. Chem.* **2008**, *23*, 37.
- (15) Vinsova, J.; Horak, V.; Buchta, V.; Kaustova, J. Highly lipophilic benzoxazoles with potential antibacterial activity. *Molecules* **2005**, *10*, 783.
- (16) Weidner-Wells, M. A.; Ohemeng, K. A.; Nguyen, V. N.; Fraga-Spano, S.; Macielag, M. J.; Werblood, H. M.; Folenó, B. D.; Webb, G. C.; Barrett, J. F.; Hlasta, D. J. Amidino benzimidazole inhibitors of bacterial two-component systems. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1545–1548.
- (17) Klimesova, V.; Koci, J.; Waisser, K.; Kaustova, J.; Mollmann, U. Preparation and in vitro evaluation of benzylsulfanyl benzoxazole derivatives as potential antituberculosis agents. *Eur. J. Med. Chem.* **2009**, *44*, 2286.
- (18) Kumar, D.; Jacob, M. R.; Reynolds, M. B.; Kerwin, S. M. Synthesis and evaluation of anticancer benzoxazole and benzimidazoles related to UK-1. *Bioorg. Med. Chem.* **2002**, *10*, 3997–4004.
- (19) Tipparaju, S. K.; Joyasawal, S.; Pieroni, M.; Kaiser, M.; Brun, R.; Kozokowski, A. P. In pursuit of natural product leads: Synthesis and biological evaluation of 2-[3-hydroxy-2-[(3-hydroxypyridine-2-carbonyl)amino]phenyl]benzoxazole-4-carboxylic acid (A-33853) and its analogues: Discovery of *N*-(2-Benzoxazol-2-ylphenyl)benzamides as novel antileishmanial chemotypes. *J. Med. Chem.* **2008**, *51*, 7344–7347.
- (20) Surleraux, D. L. N. G.; de Kock, H. A.; Verschuere, W. G.; Pille, G. M. E.; Maes, L. J. R.; Peeters, A.; Vendeville, S.; De Meyer, S.; Azijn, H.; Pauwels, R.; de Bethune, M.-P.; King, N. M.; Jeyabalan, M. P.; Schiffer, C. A.; Wigerinck, P. B. T. P. Design of HIV-1 protease inhibitors active on multidrug-resistant virus. *J. Med. Chem.* **2005**, *48*, 1965–1973.
- (21) Seerden, J.-P. G.; Ionescu, G. L.; Leguijt, R.; Saccavini, C.; Gelens, E.; Dros, B.; Vrenken, T. W.; Molema, G.; Kamps, J. A. A. M.; Kellogg, R. M. Syntheses and structure–activity relationships for some triazolyl p38 α MAPK inhibitors. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 1352–1357.
- (22) Cox, C. D.; Breslin, M. J.; Whitman, D. B.; Schreier, J. D.; McGaughey, G. B.; Bogusky, M. J.; Roecker, A. J.; Mercer, S. P.; Bednar, R. A.; Lemaire, W.; Bruno, J. G.; Reiss, D. R.; Harrell, C. M.; Murphy, K. L.; Garson, S. L.; Doran, S. M.; Prueksaritanont, T.; Anderson, W. B.; Tang, C.; Roller, S.; Cabalu, T. D.; Cui, D.; Hartman, G. D.; Young, S. D.; Koblan, K. S.; Winrow, C. J.; Renger, J. J.; Coleman, P. J. Discovery of the dual orexin receptor antagonist [(7R)-4-(5-Chloro-1,3-benzoxazol-2-yl)-7-methyl-1,4-diazepan-1-yl][5-methyl-2-(2H-1,2,3-triazol-2-yl) phenyl]methanone (MK-4305) for the treatment of insomnia. *J. Med. Chem.* **2010**, *53*, 5320–5332.
- (23) Baxter, C. A.; Cleator, E.; Brands, K. M. J.; Edwards, J. S.; Reamer, R. A.; Sheen, F. J.; Stewart, G. W.; Strotman, N. A.; Wallace, D. J. The first large scale synthesis of MK-4305: A dual orexin receptor antagonist for the treatment of sleep disorder. *Org. Process Res. Dev.* **2011**, *15*, 367–375.
- (24) Jayanna, N. D.; Vagdevi, H. M.; Dharshan, J. C.; Kekuda, T. R. P.; Hanumanthappa, B. C.; Gowdarshivannanavar, B. C. Synthesis and biological evaluation of novel 5,7-Dichloro-1,3-benzoxazole derivatives. *J. Chem.* **2013**, No. 864385.

- (25) Janardhan, S.; Sarangapani, M.; Balaswamy, G. Synthesis and characterization of substituted 5-([1,2,4]triazolo[3,4-B][1,3,4]-thiadiazol-3-yl)-1,3-benzoxazole derivatives as antimicrobial agents. *Sci. Rev. Chem. Commun.* **2012**, *2* (4), 572–579.
- (26) (a) Goikhman, R.; Jacques, T. L.; Sames, D. C–H bonds as ubiquitous functionality: A general approach to complex arylated pyrazoles via sequential regioselective C-arylation and N-alkylation enabled by SEM-group transposition. *J. Am. Chem. Soc.* **2009**, *131* (8), 3042–3048.
- (27) (a) Ellman, J. A. The direct approach. *Science* **2007**, 1131–1132. (b) Labinger, J. A.; Bercaw, J. E. Understanding and exploiting C–H bond activation. *Nature* **2002**, *417*, 507–514. (c) Giri, R.; Shi, B.-F.; Engle, K. M.; Maugel, N.; Yu, J.-Q. Transition metal-catalyzed C–H activation reactions: diastereoselectivity and enantioselectivity. *Chem. Soc. Rev.* **2009**, *38*, 3242–3272. (d) Wencel-Delord, J.; Droge, T.; Liu, F.; Glorius, F. Towards mild metal-catalyzed C–H bond activation. *Chem. Soc. Rev.* **2011**, *40*, 4740–4761.
- (28) (a) Rousseaux, S.; Liégault, B.; Fagnou, K. C–H functionalization: A new strategy for the synthesis of biologically active natural products. *Modern Tools for the Synthesis of Complex Bioactive Molecules*; Cossy, J., Arseniyadis, S., Eds.; Wiley-VCH: Hoboken, NJ, 2012; pp 1–32. (b) Chen, X.; Hao, X.-S.; Goodhue, C. E.; Yu, J.-Q. Cu(II)-catalyzed functionalizations of aryl C–H bonds using O₂ as an oxidant. *J. Am. Chem. Soc.* **2006**, *128*, 6790–6791. (c) Shang, M.; Sun, S.-Z.; Dai, H.-X.; Yu, J.-Q. Cu(II)-mediated C–H amidation and amination of arenes: Exceptional compatibility with heterocycles. *J. Am. Chem. Soc.* **2014**, *136*, 3354–3357.
- (29) (a) Wencel-Delord, J.; Glorius, F. C–H bond activation enables the rapid construction and late-stage diversification of functional molecules. *Nat. Chem.* **2013**, *5*, 369–375. (b) Gutekunst, W. R.; Baran, P. S. C–H functionalization logic in total synthesis. *Chem. Soc. Rev.* **2011**, *40*, 1976–1991.
- (30) (a) Berciano, B. P.; Lebrequier, S.; Besselievre, F.; Piguel, S. 1,1-Dibromo-1-alkenes as valuable partners in the copper-catalyzed direct alkynylation of azoles. *Org. Lett.* **2010**, *12* (18), 4038–4041. (b) Besselievre, F.; Piguel, S. Copper as a powerful catalyst in the direct alkynylation of azoles. *Angew. Chem., Int. Ed.* **2009**, *48*, 9553–9556.
- (31) (a) Dudnik, A. S.; Gevorgyan, V. Formal inverse Sonogashira reaction: direct alkynylation of arenes and heterocycles with alkynyl halides. *Angew. Chem., Int. Ed.* **2010**, *49*, 2096–2098. (b) Kawano, T.; Matsuyama, N.; Hirano, K.; Satoh, T.; Miura, M. Room temperature direct alkynylation of 1,3,4-oxadiazoles with alkynyl bromides under copper catalysis. *J. Org. Chem.* **2010**, 1764–1766.
- (32) Ramirez, F.; Desai, N. B.; Kelvie, N. Mc. A new synthesis of 1,1-dibromoolefins via phosphine-dibromoethylenes. The reaction of triphenylphosphine with carbon tetrabromide. *J. Am. Chem. Soc.* **1962**, *84*, 1745.
- (33) Chakraborty, A.; Dey, S.; Sawoo, S.; Adarsh, N. N.; Sarkar, A. Regioselective 1,3-dipolar cycloaddition reaction of azides with alkoxy alkynyl Fischer carbene complexes. *Organometallics* **2010**, *29*, 6619–6622.
- (34) Do, H.-Q.; Daugulis, O. Copper-catalyzed arylation of heterocycle C–H bonds. *J. Am. Chem. Soc.* **2007**, *129*, 12404.
- (35) Ley, S. V.; Thomas, A. W. Modern synthetic methods for copper-mediated C(aryl)–O, C(aryl)–N, and C(aryl)–S bond formation. *Angew. Chem., Int. Ed.* **2003**, *42*, 5400–5449.
- (36) Zhang, S.-L.; Liu, L.; Fu, Y.; Guo, Q.-X. Theoretical study on copper(I)-catalyzed cross-coupling between aryl halides and amides. *Organometallics* **2007**, *26*, 4546–4554.
- (37) Altman, R. A.; Koval, E. D.; Buchwald, S. L. Copper-catalyzed N-arylation of imidazoles and benzimidazoles. *J. Org. Chem.* **2007**, *72*, 6190–6199.
- (38) Patton, T.; Barrett, J.; Brennan, J.; Moran, N. Use of a spectrophotometric bioassay for determination of microbial sensitivity to manuka honey. *J. Microbiol. Methods* **2006**, *64*, 84–95.
- (39) Rao, M. L. N.; Jadhav, D. N.; Dasgupta, P. Pd-catalyzed domino synthesis of internal alkynes using triarylbismuths as multicoupling organometallic nucleophiles. *Org. Lett.* **2010**, *12*, 9.
- (40) Michaelides, I. N.; Darses, B.; Dixon, D. J. Acid-catalyzed synthesis of bicyclo[3.n.1]alkenediones. *Org. Lett.* **2011**, *13*, 4.
- (41) Galamb, V.; Gopal, M.; Alper, H. Synthesis of diynes, α,β -unsaturated monoacids, and diacids by the selective palladium(0)-catalyzed and phase transfer catalyzed reactions of vinylic dibromides. *Organometallics* **1983**, *2* (7), 801–805.
- (42) Ankati, H.; Biehl, E. Microwave-assisted benzyne-click chemistry: Preparation of 1H-benzo[d][1,2,3]triazoles. *Tetrahedron Lett.* **2009**, *50*, 4677–4682.
- (43) Stefely, J. A.; Palchadhuri, R.; Miller, P. A.; Peterson, R. J.; Moraski, G. C.; Hergenrother, P. J.; Miller, M. J. N-((1-Benzyl-1H-1,2,3-triazol-4-yl)methyl)arylamide as a new scaffold that provides rapid access to antimicrotubule agents: Synthesis and evaluation of antiproliferative activity against select cancer cell lines. *J. Med. Chem.* **2010**, *53*, 3389–3395.