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Synthesis and Antimicrobial Screening of Novel 5-(2-((1H-1,2,3-triazol-4-yl) methoxy)-2-phenylethyl)-3-methyl-4-nitroisoxazole Derivatives

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ABSTRACT

*A library of novel bis-heterocycles encompassing isoxazole and 1,2,3-triazoles were synthesized by click chemistry approach and the structures of all newly synthesized compounds were confirmed by IR, NMR and MASS spectroscopic techniques. The synthesized final compounds (**6a-m**) have been tested for their antimicrobial activity. Results showed that compounds **6m**, **6i**, **6f** and **6h** posses significant antimicrobial activity.*

Key words: Isoxazole, 1,2,3-triazoles, click chemistry and antimicrobial.

INTRODUCTION

The chemistry of heterocycles lies at the heart of drug discovery. Among a wide variety of heterocycles that have been explored for developing pharmaceutically important molecules, compounds bearing the isoxazole moiety are endowed with various types of pharmacological activities. The isoxazole unit constitutes an easily accessible nucleus and displays a wide range of organic reactivities, and could be used as effective means of preparing new molecular scaffolds. Isoxazoles have also been repeatedly shown as useful synthons in organic synthesis. Some derivatives of isoxazole exhibit a variety of pharmacological activities such as analgesic[1], anti-HIV[2], anti-microbial[3], CNS-active[4] and anti-inflammatory activity[5].

On the other hand, 1,2,3-triazole ring system has been the center of numerous studies due to its importance in industrially attractive materials, such as anticorrosive agents, dyes, photographic materials, photo stabilizers and agrochemicals[6]. Even though the 1,2,3-triazole structural moiety does not occur in nature, it may exhibit biological activities and there are many examples in the literature including anti-Gram positive bacterial[7, 8], anti-HIV[9], anti-allergic[10] [11], β-lactamase inhibitory[12], anti-convulsant[13], selective β3 adrenergic receptor agonism[14], anti-tuberculosis[15] activities.

Considering the biological importance of isoxazoles and 1,2,3-triazoles as antimicrobial agents, we aim to conjugate these two key ligands under one construct. We herein reporting, the synthesis of isoxazole and 1,2,3-triazoles based heterocycles and their in vivo antimicrobial activity.

MATERIALS AND METHODS

Melting points were determined in open capillaries and are uncorrected. ^{1}H-Nuclear Magnetic Resonance (NMR) spectra were recorded on Bruker AV 300 and 400 MHz instruments, in $CDCl_3$ using TMS as an internal standard. Chemical shifts are given in (δ) ppm and coupling constants (*J*) are given in Hz. Combinations of the following abbreviations are used to describe NMR spectra: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet. Thin layer chromatography (TLC) was carried out on aluminium sheets coated with silica gel 60 F_{254} (Merck, 1.05554) and the spots were visualized with UV light at 254 nm or alternatively by staining with aqueous basic potassium permanganate. Flash column chromatography was performed using silica gel (Merck, 60A, 100-200 mesh). Commercially available reagents were used as supplied and some of them were distilled before use. All reactions were performed in oven dried glassware. All solvents were removed by evaporation in below 45 °C under reduced pressure.

BACTERIAL AND FUNGAL STRAINS

Three Gram-negative strains, that is, *Escherichia coli*, *Proteus vulgaris* and *Klebsiella pneumoniae* and three Gram-positive bacterial strains, that is, *Enterobacter faecalis*, *Bacillus subtilis* and *Bacillus megatherium* were procured from American type culture collection, USA. Fungal strains *Candida albucance*, *Fusarium oxysporium*, *Dreschleria halide* and *Colletotrichum falcatum* were collected from department of biotechnology, chaitanya postgraduate college (autonomous), kishanpura, hanamkonda, warangal-506001, telangana, India. All bacterial strains stored at -80 ^oC were streaked on Luria-Bertani (LB) agar plates (Hi-media Laboratories, Mumbai, India) and incubated at 37 ^oC for 20 to 24 hrs. A few isolated colonies were selected from each plate and suspended in 5 ml of LB broth in sterile culture vessel. The vessel was plugged with cotton and incubated with gentle shaking (140 rpm) at 37 ^oC for 20 hrs.

PREPARATION OF INOCULUMS

By the standard method of inoculation (Bauer et al., 1966), an inoculationg loop was touched each of four or five well isolated colonies of the same morphological type and inoculum was inoculated into 5ml of nutrient broth. The broth cultures were allowed to incubate at 37 ^oC for 24 hrs until a slight visible turbidity appeared. The turbidity of actively growing broth cultures was then adjusted with broth to obtain a half of MC Farland standard ($1x10^8$ to $5x10^8$ cfu/mL). This was used as starting inoculums for the assay.

ANTIBACTERIAL AND ANTIFUNGAL ASSAY

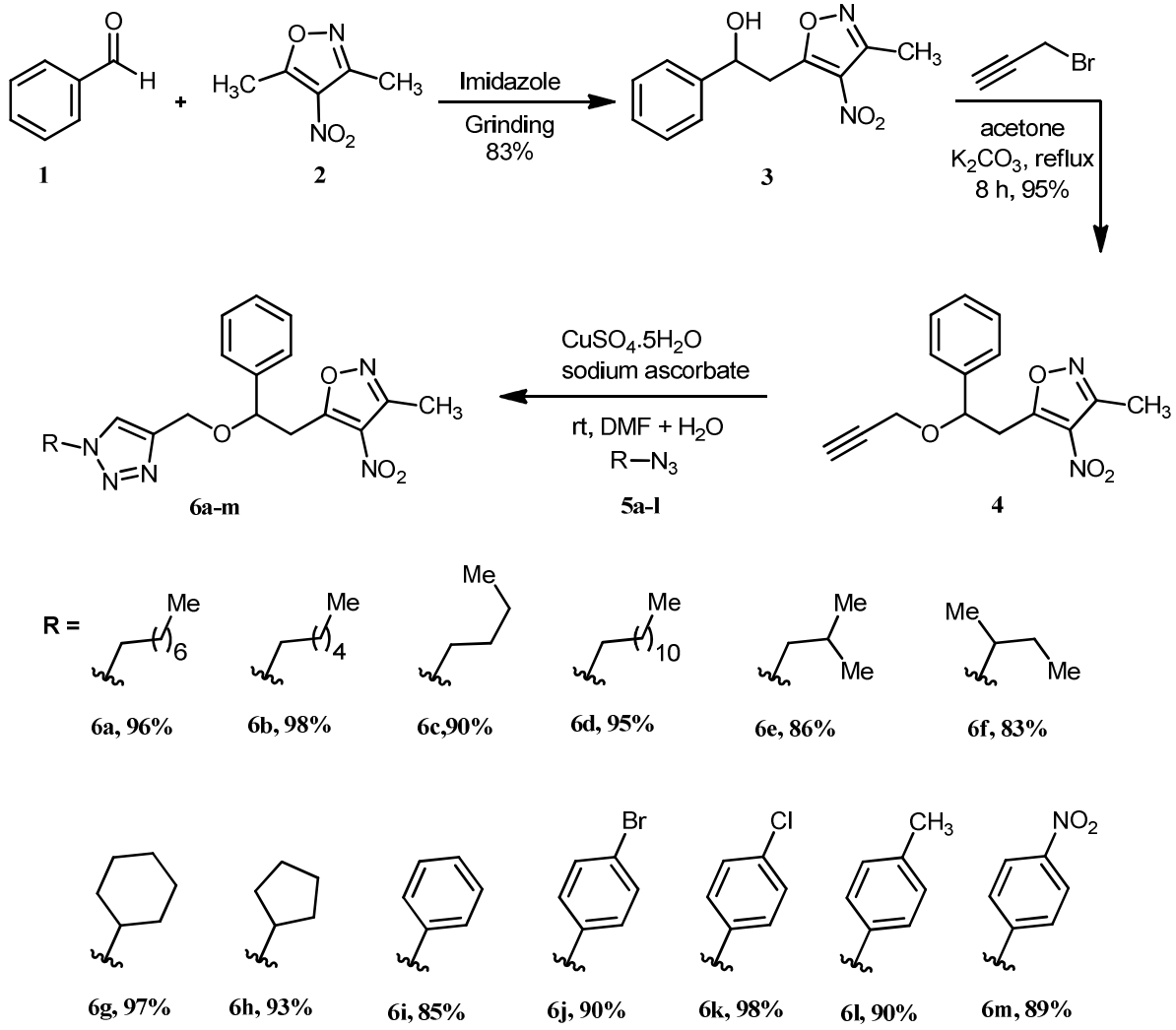
A standardized 1 to 2 x 10^7 cfu/ml 0.5 MC Farland standards was introduced onto the surface of sterile agar plate and evenly distributed the inoculums by using a sterile glass spreader. Simultaneously 8 mm wells were cut from the plate using a sterile cork borer. 50 µL of synthesized compounds at required concentrations was introduced into each well. The agar plates were incubated aerobically at 37 ^oC. After 24 hrs the inhibition zones were measured with a ruler and compared with the control well containing only DMSO.

RESULTS AND DISCUSSION

Chemistry

The general route for the synthesis of the target isoxazole based regioselective 1,4-disubstituted 1,2,3-triazoles was achieved by a 3-step protocol, as depicted in scheme 1. Compound **3** was obtained gently grinding the mixture of 3,5-dimethyl-4-nitroisoxazole (**2**), benzaldehyde (**1**) and Imidazole by hand using a motar and pestle. The *O*-alkylation of compound **3** was carried out by refluxing it with propargyl bromide and potassium carbonate in dry acetone under N_2 atmosphere for 8 hours to afford 3-methyl-4-nitro-5-(2-phenyl-2-(prop-2-yn-1-yloxy)ethyl)isoxazole (**4**) with 95% yield. This was analyzed by ^{1}H NMR spectroscopy, which showed characteristic singlets at δ 4.65, δ 2.70 due to the presence of O-CH_2 and ≡CH. With this evidence, it is confirmed that the resulting product was 3-methyl-4-nitro-5-(2-phenyl-2-(prop-2-yn-1-yloxy)ethyl)isoxazole (**4**). Then various aliphatic and aromatic azides (**5a-m**) were synthesized by utilizing literature protocols [16]. To prepare alkyl azides, corresponding alkyl halide was heated at 80-90 °C with NaN_3 in DMF. Aromatic azides were prepared from different substituted anilines using diazotization followed by treatment with NaN_3.

Scheme-1: Synthesis of isoxazole based 1,4-disubstituted 1,2,3-triazoles



According to Huisgen's [2+3] cycloaddition reaction [17], the targeted isoxazole based regioselective 1,4-disubstituted 1,2,3-triazole derivatives (**6a-m**) were synthesized by the cycloaddition reaction of 3-methyl-4-nitro-5-(2-phenyl-2-(prop-2-yn-1-yloxy)ethyl)isoxazole (**4**), with various aromatic and aliphatic azides (**5a-m**). In ^{1}H NMR spectra peak at δ 7.62-8.42 as singlet indicates the formation of 1,2,3-triazoles. The chemical structures of all target compounds were characterized by using ^{1}H NMR, ^{13}C NMR and MASS spectral analytical techniques.

BIOLOGY

Anti-bacterial activity

All the synthesized final compounds were screened for their antibacterial activities against three Gram-negative bacterial strains, that is, *E.coli*, *Proteus vulgaris*, *Enterobacter faecalis* and three Gram-positive bacterial strains, that is, *Enterobacter faecalis*, *Bacillus subtilis* and *Bacillus megatherium.* The antibacterial activity was determined by measuring the diameter of the zone of inhibition. The results of the zone of inhibition screening are summarized in table 1.

These novel derivatives (**6a-m**) demonstrated varying antibacterial activities against different strains. Compound **6m** possessing nitro functionality at 4-position of benzene ring on triazole nucleus depicted the significant zone of inhibition of 5.55, 10.18, 6.21, 18.82, 12.54 and 9.28 mm at 600 mg/ml concentration against *E.coli*, *Proteus vulgaris*, *Enterobacter faecalis*, *Enterobacter faecalis*, *Bacillus subtilis* and *Bacillus megatherium* respectively.

Compound **6i** possessing phenyl ring on triazoles nucleus showed significantly potent antibacterial activity against all tested bacterial strains. Compounds **6f** and **6h** having the aliphatic substitutions like sec-butyl and cyclopentyl groups on triazole nucleus exhibited good antibacterial activities (Table 1). Compounds **6l**, **6k**, **6j** and **6a** showed moderate activity against all tested bacterial strains. Compounds **6m**, **6i**, **6f** and **6h** were found to possess even better antibacterial activities than the standard antibiotic Streptomycin.

Table 1: Anti-bacterial activity of isoxazole based 1,4-disubstituted 1,2,3-triazoles

Compound	Conc. µg/ml	Zone of inhibition (*in mm*)					
		Gram-negative			Gram-positive		
		E.coli	*Proteus vulgaris*	*Klebsiella pnemonia*	*Enterobacter faecalis*	*Bacillus subtilis*	*Bacillus megatherium*
6a	600	2.01	6.21	2.01	10.25	7.07	4.58
	900	4.01	12.09	4.54	16.24	14.48	10.80
6b	600	1.86	6.22	1.98	NA	6.58	4.07
	900	4.01	11.48	4.21	NA	13.64	10.08
6c	600	1.25	4.05	1.01	NA	5.58	NA
	900	3.20	9.05	2.58	NA	12.86	NA
6d	600	NA	NA	1.01	8.03	4.45	3.08
	900	NA	NA	2.59	13.03	12.08	8.45
6e	600	NA	NA	NA	NA	4.05	NA
	900	NA	NA	NA	NA	11.01	NA
6f	600	3.88	9.01	4.25	16.01	11.54	8.01
	900	6.48	19.14	8.27	24.58	21.52	16.84
6g	600	1.72	NA	NA	9.07	6.05	3.76
	900	4.42	NA	NA	14.08	13.11	9.81
6h	600	3.25	8.02	3.85	13.21	9.56	7.01
	900	6.01	16.18	7.54	22.02	19.06	14.80
6i	600	4.61	9.48	5.25	16.48	12.01	8.45
	900	8.54	20.13	9.09	25.01	22.01	17.43
6j	600	2.25	6.48	2.24	11.07	7.48	5.49
	900	4.48	13.42	5.01	17.72	15.01	11.09
6k	600	2.88	7.01	2.98	11.58	8.01	6.06
	900	5.01	6.14	5.48	18.42	15.52	12.02
6l	600	3.01	7.48	3.24	12.21	8.46	6.48
	900	5.48	15.06	6.52	20.34	17.01	13.08
6m	600	5.55	10.18	6.21	18.82	12.54	9.28
	900	9.41	22.18	10.41	26.34	23.22	18.42
Standard	600	3.08	8.94	3.34	12.84	8.47	6.28
	900	6.18	16.14	6.59	20.84	16.23	12.86

Standard: Streptomycin

Anti-fungal activity

These newly synthesized isoxazole based 1,2,3-triazoles were also screened for their anti-fungal activity against microorganisms, the fungus, *Candida albucance*, *Fusarium oxysporium*, *Dreschleria halide* and *Colletotrichum falcatum*. Compounds **6f**, **6i** and **6m** were found to have improved antifungal activities 8.48, 8.02 and 7.42 mm respectively than the standard drug Itrazole 5.65 mm at concentration 600 µg/ml shown in table 2. Compounds **6h** and **6l** were exhibited good antifungal activities 5.48 and 5.08 mm at concentration 600 µg/ml respectively. *Colletotrichum falcatum* was shown resistant against compounds **6g** and **6j**. *Dreschleria halide* was shown resistant against compounds **6c**, **6d** and **6j**. Compound **6e** was found inactive against *Candida albucance* and *Fusarium oxysporium*.

Table 2: Anti-fungal activity of isoxazole based 1,4-disubstituted 1,2,3-triazoles

Compound	Conc. µg/ml	Zone of inhibition *(in mm)*			
		Candida albicans	*Fusarium oxysporium*	*Dreschleria halides*	*Colletotrichum falcatum*
6a	600	3.34	8.36	5.38	6.58
	900	10.21	15.36	11.26	14.86
6b	600	3.01	8.26	5.20	6.02
	900	9.82	15.08	11.01	14.08
6c	600	2.02	8.06	NA	5.42
	900	8.48	14.89	NA	13.02
6d	600	1.48	7.09	NA	4.06
	900	8.07	13.06	NA	13.02
6e	600	NA	NA	2.02	4.86
	900	NA	NA	8.48	12.42
6f	600	8.48	15.28	12.48	15.23
	900	17.42	26.24	21.28	23.26
6g	600	2.42	7.56	NA	NA
	900	9.06	13.42	NA	NA
6h	600	5.48	11.02	8.07	9.03
	900	13.02	20.08	15.04	18.04
6i	600	8.02	14.48	12.02	14.82
	900	16.48	25.52	20.24	22.27
6j	600	3.85	9.01	6.08	NA
	900	10.82	16.82	11.96	NA
6k	600	4.01	9.36	6.42	7.26
	900	11.42	17.26	12.08	15.24
6l	600	5.08	10.48	7.43	8.52
	900	12.06	19.82	14.04	17.74
6m	600	7.42	14.04	11.49	12.02
	900	16.09	24.02	18.42	21.22
Itrazole	600	5.65	10.86	7.58	9.44
	900	12.21	20.06	14.36	18.36

EXPERIMENTAL PROCEDURE AND SPECTRAL DATA

General procedure for the synthesis of 2-(3-Methyl-4-nitro-5-isoxazolyl)-1-phenyl-1-ethanol (3) [18]**.**

A mixture of 3,5-dimethyl-4-nitroisoxazole (**2**) (0.007 mol), benzaldehyde (**1**) (0.007 mol), and 10 mol % of imidazole was gently ground by hand using a motar and pestle. The progress of the reaction was monitored by TLC. The reaction mixture became sticky paste during the course of the reaction. Finally, it was diluted with 10 mL of ice-cold water. The solid separated was filtered and dried. The products were purified by recrystallization from ethyl acetate.

Yield: 83%; white solid; mp: 92-94 °C; IR (KBr): ν_{max} 3470 (OH), cm^{-1}; ^{1}H NMR spectrum (300 MHz, $CDCl_3$), δ, ppm: 7.54-7.47 (m, 5H), 4.87 (t, *J* = 7.0 Hz, 1H), 4.25 (bs, 1H), 3.41 (d, *J* = 7.0 Hz, 2H), 2.28 (s, 3H); ^{13}C NMR spectrum (75 MHz, $CDCl_3$), δ, ppm: 158.3, 152.4, 141.3, 128.3, 128.1, 127.3, 126.8, 126.4, 101.5, 80.2, 31.8, 13.0; EI-MS: *m/z* 249 [M+H]+.

General procedure for the synthesis of 3-methyl-4-nitro-5-(2-phenyl-2-(prop-2-yn-1-yloxy)ethyl)isoxazole (4).

To a well stirred solution of compound **4** (0.004 mol) in dry acetone and K_2CO_3 (0.008 mol), propargyl bromide 80% in toluene (0.008 mol) was added drop wise and the reaction mixture was refluxed for about 8 h. The progress of the reaction was monitored by TLC, the reaction mixture was cooled to room temperature and excess acetone was evaporated under reduced pressure, then diluted with water (50 mL) and extracted with ethyl acetate (3×50 mL). The combined organic layers were washed with brine (2×25 mL). The organic layer was dried over anhydrous sodium sulphate, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography using 100-200 mesh silica gel, eluted at 5% ethyl acetate in pet ether to afford 3-methyl-4-nitro-5-(2-phenyl-2-(prop-2-yn-1-yloxy)ethyl)isoxazole (**4**) as white solid.

Yield: 95%; white solid; mp: 112-114 °C; ^{1}H NMR spectrum (300 MHz, $CDCl_3$), δ, ppm: 7.57-7.53 (m, 5H), 4.88 (t, *J* = 7.0 Hz, 1H), 4.65 (s, 2H), 3.40 (d, *J* = 7.0 Hz, 2H), 2.70 (s, 1H), 2.28 (s, 3H); ^{13}C NMR spectrum (75 MHz, $CDCl_3$), δ, ppm: 158.2, 152.3, 141.4, 128.4, 128.2, 127.3, 126.2, 125.8, 102.3, 80.9, 78.6, 77.5, 57.2, 31.9, 13.3; EI-MS: *m/z* 287 [M+H]+.

General procedure for the synthesis of 5-(2-((1H-1,2,3-triazol-4-yl)methoxy)-2-phenylethyl)-3-methyl-4-nitroisoxazole (6a-m).

Propargyl derivative (**4**) (0.349 mol) is dissolved in 5 mL of aqueous DMF (50%) was added $CuSO_4$. $5H_2O$ (5 mol%) followed by sodium ascorbate (10 mol%) and azide (0.45 mmol) was added. The reaction mixture was stirred for 1 h at room temperature, monitored by TLC. After complete conversion of starting materials the reaction mixture was diluted with water (25 mL), extracted with ethyl acetate (3×25 mL). The combined organic layers were washed with brine (2×25 mL). The organic layer was dried over anhydrous sodium sulphate, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography using 100-200 mesh silica gel and ethyl acetate in pet ether to afford corresponding 1,4-disubstituted 1,2,3-triazole analogues (**6a-m**).

3-methyl-4-nitro-5-(2-((1-octyl-1H-1,2,3-triazol-4-yl)methoxy)-2-phenylethyl)isoxazole (6a).

Yield: 96%; white solid; mp: 126-128 °C; ^{1}H NMR spectrum (300 MHz, $CDCl_3$), δ, ppm: 7.65 (s, 1H), 7.56-7.52 (m, 5H), 4.85 (t, *J* = 7.0 Hz, 1H), 4.66 (s, 2H), 4.35 t (*J* = 5.6 Hz, 2H), 3.41 (d, *J* = 7.0 Hz, 2H), 2.28 (s, 3H), 1.94-1.86 m (2H), 1.33-1.25 m (10 H), 0.87 t (*J* = 5.3 Hz, 3H); ^{13}C NMR spectrum (75 MHz, $CDCl_3$), δ, ppm: 158.2, 152.3, 141.4, 130 (2C), 128.4, 128.2, 127.3, 126.2, 125.8, 102.3, 80.9, 62.3, 57.2, 32.5 (2C), 31.9, 31.6, 27.5, 23.8 (2C), 15.4, 13.3; EI-MS: *m/z* 442 [M+H]+.

5-(2-((1-hexyl-1H-1,2,3-triazol-4-yl)methoxy)-2-phenylethyl)-3-methyl-4-nitroisoxazole (6b).

Yield: 98%; white solid; mp: 118-120 °C; ^{1}H NMR spectrum (300 MHz, $CDCl_3$), δ, ppm: 7.62 (s, 1H), 7.54-7.50 (m, 5H), 4.83 (t, *J* = 7.0 Hz, 1H), 4.64 (s, 2H), 4.33 t (*J* = 5.6 Hz, 2H), 3.40 (d, *J* = 7.0 Hz, 2H), 2.26 (s, 3H), 1.94-1.84 m (2H), 1.33-1.25 m (6 H), 0.87 t (*J* = 5.3 Hz, 3H); ^{13}C NMR spectrum (75 MHz, $CDCl_3$), δ, ppm: 158.1, 152.3, 141.3, 130 (2C), 128.5, 128.2, 127.3, 126.6, 125.8, 102.3, 80.2, 62.3, 57.5, 32.6 (2C), 31.9, 23.8 (2C), 15.4, 13.3; EI-MS: *m/z* 414 [M+H]+.

5-(2-((1-butyl-1H-1,2,3-triazol-4-yl)methoxy)-2-phenylethyl)-3-methyl-4-nitroisoxazole (6c).

Yield: 90%; white solid; mp: 113-115 °C; ^{1}H NMR spectrum (300 MHz, $CDCl_3$), δ, ppm: 7.67 (s, 1H), 7.55-7.50 (m, 5H), 4.80 (t, *J* = 7.0 Hz, 1H), 4.64 (s, 2H), 4.34 t (*J* = 5.6 Hz, 2H), 3.43 (d, *J* = 7.0 Hz, 2H), 2.26 (s, 3H), 1.91-1.87 m (2H), 1.31-1.27 m (2 H), 0.89 t (*J* = 5.3 Hz, 3H); ^{13}C NMR spectrum (75 MHz, $CDCl_3$), δ, ppm: 158.5, 152.5, 141.3, 130.1 (2C), 128.7, 128.2, 127.3, 126.6, 125.5, 102.3, 80.7, 62.5, 57.2, 32.7, 31.9, 23.8, 15.4, 13.3; EI-MS: *m/z* 386 [M+H]+.

5-(2-((1-dodecyl-1H-1,2,3-triazol-4-yl)methoxy)-2-phenylethyl)-3-methyl-4-nitroisoxazole (6d).

Yield: 95%; white solid; mp: 141-143 °C; ^{1}H NMR spectrum (300 MHz, $CDCl_3$), δ, ppm: 7.71 (s, 1H), 7.57-7.52 (m, 5H), 4.87 (t, *J* = 7.2 Hz, 1H), 4.64 (s, 2H), 4.37 t (*J* = 5.6 Hz, 2H), 3.43 (d, *J* = 7.0 Hz, 2H), 2.30 (s, 3H), 1.94-1.86 m (2H), 1.36-1.20 m (18 H), 0.82 t (*J* = 5.3 Hz, 3H); ^{13}C NMR spectrum (75 MHz, $CDCl_3$), δ, ppm: 159.0, 152.7, 141.8, 130.3 (2C), 128.6, 128.7, 127.0, 126.9, 126.1, 102.1, 80.4, 62.5, 57.5, 32.5 (2C), 31.9, 31.6 (2C), 27.5, 25.1 (2C), 23.8 (2C), 15.4, 16.6, 13.3; EI-MS: *m/z* 498 [M+H]+.

5-(2-((1-isobutyl-1H-1,2,3-triazol-4-yl)methoxy)-2-phenylethyl)-3-methyl-4-nitroisoxazole (6e).

Yield: 86%; white solid; mp: 121-123 °C; ^{1}H NMR spectrum (300 MHz, $CDCl_3$), δ, ppm: 7.66 (s, 1H), 7.55-7.51 (m, 5H), 4.80 (t, *J* = 7.0 Hz, 1H), 4.64 (s, 2H), 4.19 d (*J* = 7.23 Hz, 2H), 3.43 (d, *J* = 7.0 Hz, 2H), 2.26 (s, 3H), 2.27-2.22 m (1H), 0.98 d (*J* = 6.70 Hz, 6H); ^{13}C NMR spectrum (75 MHz, $CDCl_3$), δ, ppm: 158.5, 152.5, 141.3, 130.1 (2C), 128.7, 128.2, 127.3, 126.6, 125.5, 102.3, 80.7, 62.5, 57.2, 44.8, 32.7, 29.7, 19.8 (2C); EI-MS: *m/z* 386 [M+H]+.

5-(2-((1-(sec-butyl)-1H-1,2,3-triazol-4-yl)methoxy)-2-phenylethyl)-3-methyl-4-nitroisoxazole (6f).

Yield: 83%; white solid; mp: 130-133 °C; ^{1}H NMR spectrum (300 MHz, $CDCl_3$), δ, ppm: 7.67 (s, 1H), 7.56-7.50 (m, 5H), 4.83 (t, *J* = 6.9 Hz, 1H), 4.61 (s, 2H), 4.66-4.53 m (1H), 3.43 (d, *J* = 7.0 Hz, 2H), 2.26 (s, 3H), 1.96-1.82 m (2H), 1.57 d (*J* = 6.63 Hz, 3H), 0.86 t (*J* = 7.16 Hz, 3H); ^{13}C NMR spectrum (75 MHz, $CDCl_3$), δ, ppm: 157.9, 152.3, 141.3, 130.3 (2C), 128.7, 128.3, 127.3, 126.6, 125.6, 102.3, 80.7, 62.5, 57.2, 44.8, 32.7, 44.8, 20.9, 10.4; EI-MS: *m/z* 386 [M+H]+.

5-(2-((1-cyclohexyl-1H-1,2,3-triazol-4-yl)methoxy)-2-phenylethyl)-3-methyl-4-nitroisoxazole (6g).

Yield: 97%; white solid; mp: 139-141 °C; ^{1}H NMR spectrum (300 MHz, $CDCl_3$), δ, ppm: 7.80 (s, 1H), 7.57-7.52 (m, 5H), 4.83 (t, *J* = 7.1 Hz, 1H), 4.64 (s, 2H), 4.50-4.41 m (1H), 3.40 (d, *J* = 7.0 Hz, 2H), 2.26 (s, 3H), 2.25-2.20 m

(2H), 1.96-1.91 m (2H), 1.79-1.72 m (3H), 1.53-1.40 m (2H), 1.34-1.26 m (1H); ^{13}C NMR spectrum (75 MHz, $CDCl_3$), δ, ppm: 157.8, 152.1, 141.3, 130.3 (2C), 128.5, 128.2, 127.5, 126.6, 125.8, 102.3, 80.2, 62.3, 57.5, 35.8 (2C), 32.6 (2C), 27.5 (2C), 27.4; EI-MS: *m/z* 412 [M+H]+.

5-(2-((1-cyclopentyl-1H-1,2,3-triazol-4-yl)methoxy)-2-phenylethyl)-3-methyl-4-nitroisoxazole (6h).
Yield: 93%; white solid; mp: 136-148 °C; ^{1}H NMR spectrum (300 MHz, $CDCl_3$), δ, ppm: 7.75 (s, 1H), 7.58-7.53 (m, 5H), 4.82 (t, *J* = 7.0 Hz, 1H), 4.65 (s, 2H), 4.99-4.93 m (1H), 3.41 (d, *J* = 7.0 Hz, 2H), 2.27 (s, 3H), 2.33-2.23 m (2H), 2.11-2.01 m (2H), 1.97-1.86 m (2H), 1.83-1.71 m (2H); ^{13}C NMR spectrum (75 MHz, $CDCl_3$), δ, ppm: 157.7, 152.3, 141.2, 130.0 (2C), 128.6, 128.2, 127.7, 126.6, 125.8, 102.5, 80.2, 62.3, 57.5, 46.9, 35.8, 35.6 (2C), 26.4 (2C); EI-MS: *m/z* 398 [M+H]+.

3-methyl-4-nitro-5-(2-phenyl-2-((1-phenyl-1H-1,2,3-triazol-4-yl)methoxy)ethyl)isoxazole (6i).
Yield: 85%; white solid; mp: 138-140 °C; ^{1}H NMR spectrum (300 MHz, $CDCl_3$), δ, ppm: 7.80 (s, 1H), 7.66-7.59 (4H), 7.58-7.50 (m, 6H), 4.87 (t, *J* = 6.9 Hz, 1H), 4.65 (s, 2H), 3.41 (d, *J* = 7.0 Hz, 2H), 2.27 (s, 3H); ^{13}C NMR spectrum (75 MHz, $CDCl_3$), δ, ppm: 158.2, 152.3, 141.4, 137.1, 131 (2C), 129.0 (2C), 128.4, 128.2, 127.6, 127.4, 126.2, 125.8, 120.8 (2C), 102.3, 80.5, 63.0, 31.5, 13.0; EI-MS: *m/z* 406 [M+H]+.

5-(2-((1-(4-bromophenyl)-1H-1,2,3-triazol-4-yl)methoxy)-2-phenylethyl)-3-methyl-4-nitroisoxazole (6j).
Yield: 90%; white solid; mp: 150-152 °C; ^{1}H NMR spectrum (300 MHz, $CDCl_3$), δ, ppm: 8.01 (s, 1H), 7.63-7.57 (4H), 7.56-7.52 (m, 5H), 4.86 (t, *J* = 7.1 Hz, 1H), 4.66 (s, 2H), 3.41 (d, *J* = 7.0 Hz, 2H), 2.28 (s, 3H); ^{13}C NMR spectrum (75 MHz, $CDCl_3$), δ, ppm: 158.6, 152.1, 141.4, 135.7, 131.6 (2C),130.8 (2C), 128.6, 128.2, 128.0 (2C), 127.3, 126.2, 125.8, 123.5, 102.3, 80.8, 63.1, 31.7, 13.3; EI-MS: *m/z* 435 [M+H]+.

5-(2-((1-(4-chlorophenyl)-1H-1,2,3-triazol-4-yl)methoxy)-2-phenylethyl)-3-methyl-4-nitroisoxazole (6k).
Yield: 98%; white solid; mp: 137-139 °C; ^{1}H NMR spectrum (300 MHz, $CDCl_3$), δ, ppm: 8.03 (s, 1H), 7.61-7.54 (4H), 7.51-7.46 (m, 5H), 4.88 (t, *J* = 7.0 Hz, 1H), 4.65 (s, 2H), 3.39 (d, *J* = 7.0 Hz, 2H), 2.30 (s, 3H); ^{13}C NMR spectrum (75 MHz, $CDCl_3$), δ, ppm: 158.2, 152.3, 141.4, 135.2, 134.0, 131 (2C), 128.8 (2C), 128.4, 128.2, 127.4, 126.2, 125.9, 122.2 (2C), 102.4, 80.7, 63.0, 31.6, 13.1; EI-MS: *m/z* 440 [M+H]+.

3-methyl-4-nitro-5-(2-phenyl-2-((1-(p-tolyl)-1H-1,2,3-triazol-4-yl)methoxy)ethyl)isoxazole (6l).
Yield: 90%; white solid; mp: 144-146 °C; ^{1}H NMR spectrum (300 MHz, $CDCl_3$), δ, ppm: 7.76 (s, 1H), 7.64-7.58 (4H), 7.56-7.51 (m, 6H), 4.86 (t, *J* = 6.8 Hz, 1H), 4.62 (s, 2H), 3.42 (d, *J* = 7.1 Hz, 2H), 2.30 (s, 3H), 2.43 (s, 3H); ^{13}C NMR spectrum (75 MHz, $CDCl_3$), δ, ppm: 158.0, 152.1, 141.5, 138.6, 134.8 (2C), 133.3, 131.2 (2C), 129.5 (2C), 128.4, 128.6, 127.5, 126.3, 125.8, 102.6, 80.6, 62.9, 30.9, 23.5, 13.6; EI-MS: *m/z* 420 [M+H]+.

3-methyl-4-nitro-5-(2-((1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl)methoxy)-2-phenylethyl)isoxazole (6m).
Yield: 89%; yellow solid; mp: 160-162 °C; ^{1}H NMR spectrum (300 MHz, $CDCl_3$), δ, ppm: 8.42 (d, *J* = 9.0 Hz, 2H), 8.19 (s, 1H), 8.00 d (*J* = 8.50 Hz, 2H), 7.56-7.50 (m, 5H), 4.86 (t, *J* = 7.1 Hz, 1H), 4.64 (s, 2H), 3.42 (d, *J* = 7.0 Hz, 2H), 2.28 (s, 3H); ^{13}C NMR spectrum (75 MHz, $CDCl_3$), δ, ppm: 158.2, 152.3, 148.3, 144.6, 141.4, 131 (2C), 128.4, 128.2, 127.3, 126.2, 125.8, 124.5 (2C), 120.4 (2C), 102.3, 80.6, 63.0, 31.5, 13.0; EI-MS: *m/z* 451 [M+H]+.

CONCLUSION

A group of novel 5-(2-((1H-1,2,3-triazol-4-yl)methoxy)-2-phenylethyl)-3-methyl-4-nitroisoxazoles (**6a-m**) have been synthesized in good yields using click chemistry approach and characterized by different spectroscopic techniques. All the newly synthesized molecules showed moderate to good antibacterial and antifungal activities. Among all compounds **6m**, **6i**, **6f** and **6h** are found to shown maximum activity. These results gave us positive encouragement to develop further novel chemical entities towards challenging bioactive agents.

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