Asian Journal of Chemistry; Vol. 29, No. 12 (2017), 2660-2664

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2017.20781

Synthesis of Isoxazoles *via* 1,3-Dipolar Cycloaddition Reactions: Pharmacological Screening for Their Antioxidant and Antimicrobial Activities

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Received: 15 May 2017; *Accepted*: 28 July 2017; *Published online*: 30 October 2017; AJC-18611

In the present study, we report the synthesis of series of novel substituted isoxazoles *via* 1,3-dipolar cycloaddition reaction. Nitrile oxides generated by the catalytic dehydrogenation of aldoximes by chloramine-T, were trapped *in situ* by 4-(furan-2-yl)but-3-en-2-one to obtain an isomeric mixture of isoxazoles. The structure of the new isoxazoles were elucidated by spectral and elemental analyses. The synthesized isoxazoles have been screened *in vitro* for their DPPH radical scavenging abilities and antimicrobial activities. Amongst the synthesized series, compound 1-(3-(4-chlorophenyl)-4-(furan-2-yl)-4,5-dihydroisoxazol-5-yl)ethanone (**4f**) exhibited excellent radical scavenging and antimicrobial susceptibilities.

Keywords: Antimicrobial, Antioxidant, Cycloaddition, Dipolar, Radical scavenging.

INTRODUCTION

Oxidative stress on a cell due to high concentration of ROS can lead to a variety of disorders including cancer, neurodegenerative disorder, atherosclerosis and aging [1]. An interest in antioxidant activity of small molecules, to prevent the deleterious effects caused by free radicals is attracting the attention of the wider research community. Free radicals are arising due to the oxidative stress resulting from an imbalance between free radical generation and their quenching [2]. Five membered heterocycles are important class of compounds in bioorganic chemistry, among them isoxazoles occupies a prime position due to their versatile synthetic and biological applications [3]. The efforts have been made to the development of general and versatile synthetic methodologies for the synthesis of isoxazoline. However, the classical method employed for the synthesis of isoxazoline involves 1,3-dipolar cycloaddition reactions of nitrile oxides to alkenes [4-6]. Rai *et al.* [7] developed new method for generating nitrile oxides involving oxidative dehydrogenation of aldoximes using oxidants such as chloramine-T and mercuric acetate.

Isoxazoles and their derivatives are known to exhibit broad spectrum of biological activities, such as anticancer [8], antiarthritic [9], antimicrobial [10], antinociceptive [11], selective agonists at human cloned dopamine D4 receptors [12], antioxidant [13], $GABA_A$ antagonist [14] and COX-2 inhibitor [15] properties. With these observations in the back-ground and in search of new potent antimicrobial and antioxidant small molecules, we herein report the synthesis of new isoxazole derivatives by 1,3-dipolar cycloaddition reactions and *in vitro* evaluation results of their antimicrobial and antioxidant activities.

EXPERIMENTAL

Melting points were determined by an open capillary tube method and are uncorrected. Purity of the compounds was checked on thin layer chromatography (TLC). ^{1}H NMR and ^{13}C NMR spectra were recorded on Agilent-NMR 400 M Hz and 100 M Hz spectrometer respectively. The solvent $CDCl_3$ with TMS as an internal standard was used to record the spectra. The chemical shifts are expressed in δ ppm. Mass spectra were obtained on ESI/APCI-Hybrid Quadrupole, Synapt G2 HDMS ACQUITY UPLC model spectrometer. Elemental analysis was obtained on a Thermo Finnigan Flash EA 1112 CHN analyzer.

Initially, the precursor aromatic aldoximes **2**(**a-g**) were prepared by reacting aromatic aldehydes **1**(**a-g**) with hydroxylamine hydrochloride in the presence of sodium acetate in ethanol as a solvent. Then, the reaction of 4-(furan-2-yl)but-3-en-2-one (**3**) as dipolarophile moiety with aldoximes **2**(**a-g**) and chloramine-T in ethyl alcohol under reflux conditions produced a mixture of 3-aryl-1-(4-(furan-2-yl)-4,5-dihydroisoxazol-5-yl)ethanones **4**(**a-g**) (major product) and 3-aryl-1-(5-(furan-2-yl)-4,5-dihydroisoxazol-5-yl)ethanones **5**(**a-g**) (minor product). Chloramine-T (CAT) served as an effective catalytic dehydrogenating agent for the conversion of

aldoximes to nitrile oxides [16], also effective aziridination agent for alkenes [17]. Oxidative dehydrogenation of aldoximes **2**(**a-g**) by chloramine-T afforded nitrile oxides, which was *in situ* trapped by 4-(furan-2-yl)but-3-en-2-one (**3**) to obtain isormeric mixture of products (Fig. 1).

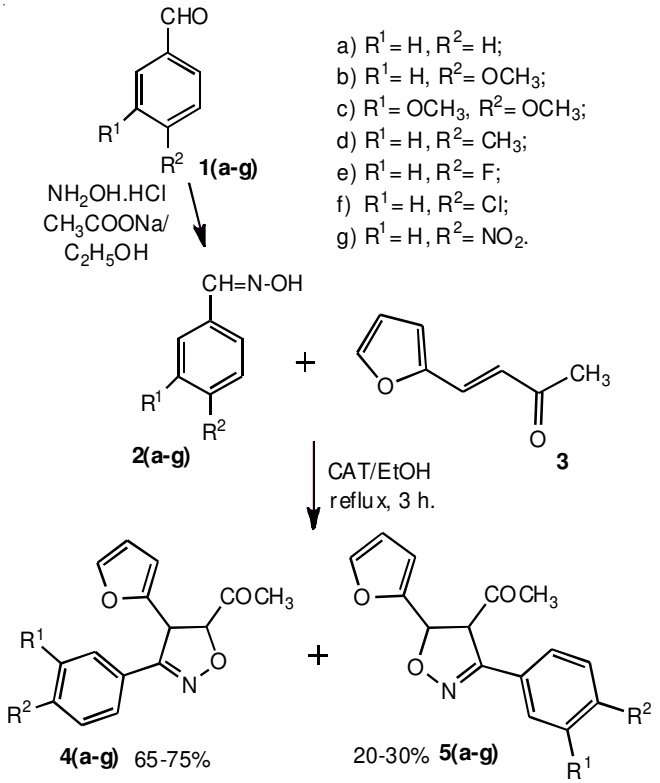

Fig. 1. Schematic diagram for the synthesis route for isoxazole derivatives

Synthesis of oximes 2(a-g): A solution of colourless phenyl hydrazine hydrochloride (0.5 g) and crystallized sodium acetate (0.8 g) in distilled water (10 mL) was mixed with solution of aldehydes **3(a-g)** (0.5 g) in ethyl alcohol. The mixture was then warmed for 5-10 min and cooled in ice water. The crystals formed were filtered, washed with a little cold water and recrystallized from ethanol.

General procedure for synthesis of isoxazoles 4(**a-g**), **5**(**a-g**). A mixture of aldoximes **2**(**a-g**) (5 mmol), 4-(furan-2-yl)but-3-en-2-one (**3**) (5 mmol) and chloramine-T trihydrate (7.5 mmol) was refluxed on a water bath for 3-4 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the salts formed were filtered off and the solvent was evaporated in vacuum. The residual mass was extracted into ether (1 × 25 mL), washed successively with water (3 × 20 mL), 5 % sodium hydroxide (2 × 10 mL), brine solution (1 × 15 mL) and dried over anhydrous sodium sulphate. Evaporation of the solvent afforded crude oil, which gave one major spot corresponding to the products 3-aryl-1-(4-(furan-2-yl)-4,5-dihydroisoxazol-5-yl)ethanones **4**(**a-g**) and a minor spot corresponding to the product 3-aryl-1-(5-(furan-2-yl)-4,5-dihydroisoxazol-5-yl)ethanones **5**(**a-g**) in TLC. The products were separated by HPLC.

1-[4-(Furan-2-yl)-3-phenyl-4,5-dihydroisoxazol-5-yl]ethanone (4a): 4-(Furan-2-yl)but-3-en-2-one (**3**) (5 mmol) and benzaldehyde oxime (**2a**) (5 mol) as pale yellow oil in 68 % yield. ^{1}H NMR ($CDCl_3$; δ ppm): 2.136 (s, 3H, CH_3), 3.658 (d, 1H, *J* = *7.2* Hz, 4-H), 4.452 (d, 1H, *J* = 7.0 Hz, 5-H), 6.102-6.387 (m, 2H, Ar-H), 7.481-7.782 (m, 6H, Ar-H); ^{13}C NMR ($CDCl_3$; δ ppm): 24.32 (1C, CH_3), 30.60 (1C, 4-C), 84.34 (1C, 5-C), 105.10 (1C), 110.12 (1C), 128.30 (1C), 128.36 (1C), 128.60 (1C), 128.65 (1C), 131.40 (1C), 136.80 (1C), 141.56 (1C), 151.70 (1C), 162.80 (1C, 3-C), 203.86 (1C, C=O). MS (*m/z*): 255.09 (M+, 100); Anal. Calcd. for $C_{15}H_{13}NO_3$ (%): C, 70.58; H, 5.13; N, 5.49; Found: C, 70.44; H, 5.02; N, 5.36.

1-[4-(Furan-2-yl)-3-(4-methoxyphenyl)-4,5-dihydroisoxazol-5-yl]ethanone (4b): 4-(Furan-2-yl)but-3-en-2-one (**3**) (5 mmol) and 4-methoxybenzaldehye oxime (**2b**) (5 mol), as pale yellow oil in 66 % yield. ^{1}H NMR ($CDCl_3$; δ ppm): 2.140 (s, 3H, CH_3), 3.665 (d, 1H, *J* = *7.4* Hz, 4-H), 3.842 (s, 3H, OCH_3), 4.446 (d, 1H, *J* = 7.1 Hz, 5-H), 6.113-6.356 (m, 2H, Ar-H), 7.184-7.756 (m, 5H, Ar-H); ^{13}C NMR ($CDCl_3$; δ ppm): 24.50 (1C, CH_3), 30.45 (1C, 4-C), 55.45 (1C), 84.55 (1C, 5-C), 105.34 (1C), 110.65 (1C), 115.32 (1C), 115.40 (1C), 126.30 (1C), 128.68 (1C), 128.74 (1C), 141.52 (1C), 151.48 (1C), 159.85 (1C), 163.12 (1C, 3-C), 203.60 (1C, C=O). MS (*m/z*): 285.10 (M+, 100); Anal. Calcd. for $C_{16}H_{15}NO_4$ (%): C, 67.36; H, 5.30; N, 4.91; Found: C, 67.25; H, 5.12; N, 4.76.

1-[3-(3,4-Dimethoxyphenyl)-4-(furan-2-yl)-4,5-dihydroisoxazol-5-yl]ethanone (4c): 4-(Furan-2-yl)but-3-en-2-one (**3**) (5 mmol) and 3,4-dimethoxybenzaldehye oxime (**2c**) (5 mol), as pale yellow oil in 74 % yield. ^{1}H NMR ($CDCl_3$; δ ppm): 2.202 (s, 3H, CH_3), 3.640 (d, 1H, *J* = *7.7* Hz, 4-H), 3.852 (s, 6H, CH_3), 4.458 (d, 1H, *J* = 7.5 Hz, 5-H), 6.121-6.370 (m, 2H, Ar-H), 7.320-7.774 (m, 4H, Ar-H); ^{13}C NMR ($CDCl_3$; δ ppm): 24.80 (1C, CH_3), 30.68 (1C, 4-C), 55.86 (1C, OCH_3), 55.90 (1C, OCH_3), 84.95 (1C, 5-C), 105.43 (1C), 110.22 (1C), 112.66 (1C), 115.31 (1C), 120.35 (1C), 121.55 (1C), 141.40 (1C), 148.45 (1C), 151.60 (1C), 152.82 (1C), 162.22 (1C, 3-C), 203.90 (1C, C=O). MS (*m/z*): 315.11 (M+, 100); Anal. Calcd. for $C_{17}H_{17}NO_5$ (%): C, 64.75; H, 5.43; N, 4.44; Found: C, 64.57; H, 5.33; N, 4.31.

1-[4-(Furan-2-yl)-3-(*p*-tolyl)-4,5-dihydroisoxazol-5-yl]-ethanone (4d): 4-(Furan-2-yl)but-3-en-2-one (**3**) (5 mmol) and 4-methyl benzaldehye oxime (**2d**) (5 mol), as pale yellow oil in 70 % yield. ^{1}H NMR ($CDCl_3$; δ ppm): 2.190 (s, 3H, CH_3), 2.324 (s, 3H, CH_3), 3.660 (d, 1H, *J* = 7.0 Hz, 4-H), 4.455 (d, 1H, *J* = *7.6* Hz, 5-H), 6.185-6.345 (m, 2H, Ar-H), 7.455-7.795 (m, 5H, Ar-H); ^{13}C NMR ($CDCl_3$; δ ppm): 21.30 (1C, CH_3), 24.50 (1C, CH_3), 30.36 (1C, 4-C), 84.08 (1C, 5-C), 105.62 (1C), 110.98 (1C), 1278.80 (1C), 127.86 (1C), 129.12 (1C), 129.16 (1C), 131.74 (1C), 141.50 (1C), 142.56 (1C), 151.44 (1C), 161.88 (1C, 3-C), 203.77 (1C, C=O). MS (*m/z*): 269.11 (M+, 100); Anal. calcd. for $C_{16}H_{15}NO_3$ (%): C, 71.36; H, 5.61; N, 5.20; Found: C, 71.25; H, 5.50; N, 5.08.

1-[3-(4-Fluorophenyl)-4-(furan-2-yl)-4,5-dihydroisoxazol-5-yl]ethanone (4e): 4-(Furan-2-yl)but-3-en-2-one (**3**) (5 mmol) and 4-fluorobenzaldehye oxime (**2e**) (5 mol), as pale yellow oil in 65 % yield. ^{1}H NMR ($CDCl_3$; δ ppm): 2.210 (s, 3H, CH_3), 3.685 (d, 1H, *J* = *7.9* Hz, 4-H), 4.474 (d, 1H, *J* = 6.9 Hz, 5-H), 6.130-6.342 (m, 2H, Ar-H), 7.526-7.780 (m, 5H, Ar-H); ^{13}C NMR ($CDCl_3$; δ ppm): 24.51 (1C, CH_3), 30.74 (1C, 4-C), 84.12 (1C, 5-C), 105.90 (1C), 111.10 (1C), 114.33 (1C), 114.38 (1C), 128.88 (1C), 128.95 (1C), 130.48 (1C),

141.32 (1C), 151.55 (1C), 162.44 (1C, 3-C), 166.80 (1C), 203.20 (1C, C=O). MS (*m/z*): 273.08 (M+, 100); Anal. Calcd. for $C_{15}H_{12}NO_3F$ (%): C, 65.93; H, 4.43; N, 5.13; Found: C, 65.81; H, 4.31; N, 5.04.

1-[3-(4-Chlorophenyl)-4-(furan-2-yl)-4,5-dihydroisoxazol-5-yl]ethanone (4f): 4-(Furan-2-yl)but-3-en-2-one (**3**) (5 mmol) and 4-chlorobenzaldehye oxime (**2f**) (5 mol), as pale yellow oil in 73 % yield ^{1}H NMR ($CDCl_3$; δ ppm): 2.155 (s, 3H, CH_3), 3.639 (d, 1H, *J* = 7.8 Hz, 4-H), 4.456 (d, 1H, *J* = *7.1* Hz, 5-H), 6.100-6.341 (m, 2H, Ar-H), 7.5301-7.793 (m, 5H, Ar-H); ^{13}C NMR ($CDCl_3$; δ ppm): 24.40 (1C, CH_3), 30.55 (1C, 4-C), 84.71 (1C, 5-C), 105.44 (1C), 110.78 (1C), 128.24 (1C), 128.31 (1C), 128.92 (1C), 128.97 (1C), 131.88 (1C), 136.41 (1C), 141.35 (1C), 151.67 (1C), 162.97 (1C, 3-C), 203.66 (1C, C=O). MS (*m/z*): 291.05 (M+2, 33), 289.05 (M+, 100); Anal. Calcd. for $C_{15}H_{12}NO_3Cl$ (%): C, 62.19; H, 4.17; N, 4.83; Found: C, 62.10; H, 4.03; N, 4.70.

1-[4-(Furan-2-yl)-3-(4-nitrophenyl)-4,5-dihydroisoxazol-5-yl]ethanone (4g): 4-(Furan-2-yl)but-3-en-2-one (**3**) (5 mmol) and 4-nitrobenzaldehye oxime (**2g**) (5 mol), as pale yellow oil in 65 % yield. ^{1}H NMR ($CDCl_3$; δ ppm): 2.148 (s, 3H, CH_3), 3.647 (d, 1H, *J* = 7.7 Hz, 4-H), 4.450 (d, 1H, *J* = 7.4 Hz, 5-H), 6.108-6.378 (m, 2H, Ar-H), 7.654-7.995 (m, 5H, Ar-H); ^{13}C NMR ($CDCl_3$; δ ppm): 24.80 (1C, CH_3), 30.32 (1C, 4-C), 84.70 (1C, 5-C), 106.02 (1C), 110.33 (1C), 127.30 (1C), 127.36 (1C), 127.84 (1C), 127.90 (1C), 140.55 (1C), 141.20 (1C), 151.14 (1C), 151.54 (1C), 163.12 (1C, 3-C), 204.22 (1C, C=O). MS (*m/z*): 300.07 (M+, 100); Anal. Calcd. for $C_{15}H_{12}N_2O_5$ (%): C, 60.00; H, 4.03; N, 9.33; Found: C, 59.90; H, 3.87; N, 9.20.

1-[5-(Furan-2-yl)-3-(4-methoxyphenyl)-4,5-dihydroisoxazol-4-yl]ethanone (5b): 4-(Furan-2-yl)but-3-en-2-one (**3**) (5 mmol) and 4-methoxybenzaldehye oxime (**2b**) (5 mol), as pale yellow oil in 26 % yield. ^{1}H NMR ($CDCl_3$; δ ppm): 2.320 (s, 3H, CH_3), 2.690 (d, 1H, *J* = 7.0 Hz, 4-H), 3.840 (s, 3H, OCH_3), 4.980 (d, 1H, *J* = 7.5 Hz, 5-H), 6.322-6.484 (m, 2H, Ar-H), 7.382-7.886 (m, 5H, Ar-H); ^{13}C NMR ($CDCl_3$; δ ppm): 27.98 (1C, CH_3), 47.65 (1C, 4-C), 55.84 (1C, OCH_3), 65.30 (1C, 5-C), 110.02 (1C), 110.55 (1C), 114.36 (1C), 114.40 (1C), 120.64 (1C), 120.70 (1C), 121.86 (1C), 140.80 (1C), 151.56 (1C), 152.84 (1C, 3-C), 161.75 (1C), 203.50 (1C, C=O). MS (*m/z*): 285.04 (M+, 100); Anal. Calcd. for $C_{16}H_{15}NO_4$ (%): C, 67.36; H, 5.30; N, 4.91; Found: C, 67.22; H, 5.22; N, 4.79.

1-[3-(4-Fluorophenyl)-5-(furan-2-yl)-4,5-dihydroisoxazol-4-yl]ethanone (5e): 4-(Furan-2-yl)but-3-en-2-one (**3**) (5 mmol) and 4-fluorobenzaldehye oxime (**2e**) (5 mol), as pale yellow oil in 24 % yield. ^{1}H NMR ($CDCl_3$; δ ppm): 2.323 (s, 3H, CH_3), 2.688 (d, 1H, *J* = 7.7 Hz, 4-H), 4.992 (d, 1H, *J* = 7.5 Hz, 5-H), 6.302-6.390 (m, 2H, Ar-H), 7.568-7.980 (m, 5H, Ar-H); ^{13}C NMR ($CDCl_3$; δ ppm): 27.80 (1C, CH_3), 46.65 (1C, 4-C), 64.86 (1C, 5-C), 109.16 (1C), 110.24 (1C), 116.33 (1C), 116.38 (1C), 124.45 (1C), 129.66 (1C), 129.69 (1C), 141.52 (1C), 152.85 (1C), 154.54 (1C, 3-C), 163.81 (1C), 203.10 (1C, C=O). MS (*m/z*): 273.02 (M+, 100); Anal. Calcd. for $C_{15}H_{12}FNO_3$ (%): C, 65.93; H, 4.43; N, 5.13; Found: C, 65.86; H, 4.27; N, 5.02.

DPPH radical scavenging assay: 1,1-Diphenylpicrylhydrazyl (DPPH) radical scavenging ability of synthesized compounds **4**(**a**-**g**) was performed by Blois method [18]. 1 mL of DPPH solution (0.1 mM in 95 % methanol) was mixed with different aliquots of test samples (25, 50, 75 and 100 µg/mL) in methanol. The mixture was shaken vigorously and allowed to stand for 20 min at room temperature. The absorbance was read against blank at 517 nm in an ELICO SL-159 UV-visible spectrophotometer. Radical scavenging potential was calculated as a percentage (I %) of DPPH decolouration using the equation:

$$I\,(\%) = \frac{A_0 - A_1}{A_0} \times 100$$

A_0 is the absorbance of the control without test compounds; A_1 is the absorbance of test compounds.

Antimicrobial activity: Antimicrobial activities of the synthesized compounds were determined as minimum inhibitory concentrations (MIC's) by serial dilution method [19]. The nutrient broth, which contains logarithmic serially two-fold diluted amount of compounds **4**(**a**-**g**) and control was inoculated with approximately 5×10^5 c.f.u of actively dividing bacteria cells. The cultures were incubated for 24 h at 37 °C for bacterial and 72 h at 37 °C for fungal stains and the growth was monitored visually. The tests were conducted in triplicates against bacterial pathogens *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus*; and against fungal stains *Aspergillus nigar, Aspergillus flavus* and *Candila albicans.* Ciprofloxacin and nystatin were used as positive controls against bacterial and fungal species, respectively; methanol was used as solvent control.

RESULTS AND DISCUSSION

^{1}H NMR, ^{13}C NMR, Mass spectra and elemental analysis provided the structural proof of the compounds **4**(**a-g**) and **5**(**a-g**). Nitrile oxides generated *in situ* by the catalytic dehydrogenation of aldoximes **2**(**a-g**) with chloramine-T were trapped *in situ* by 4-(furan-2-yl)but-3-en-2-one **3** to obtain an isomeric mixture of isoxazoles **4**(**a-g**) and **5**(**a-g**). Compound 1-(4-(furan-2-yl)-3-(4-methoxyphenyl)-4,5-dihydroisoxazol-5-yl)ethanone (**4b**) in its ^{1}H NMR spectrum, showed a singlets for three protons each at δ 2.140 and δ 3.842 ppm due to CH_3 and OCH_3 protons. The 4-H resonates as doublet at δ 3.665 (*J* = 7.4 Hz) ppm; while the 5-H appears as doublet at δ 4.446 (*J* = 7.1 Hz) ppm. Multiplets appear at δ 6.113-6.356 ppm for two protons and at δ 7.184-7.756 ppm for five protons were unambiguously assigned to aromatic protons. In the ^{13}C NMR spectrum, the carbons of newly formed isoxazole ring 4-C, 5-C and 3-C coupled at δ 30.45, 84.55 and 163.12 ppm, respectively. Signals appeared at δ 24.50, 55.45 and 203.60 ppm were due to CH_3, OCH_3 and C=O carbons. Aromatic carbons showed the signals at δ 105.34, 110.65, 115.32, 115.40, 126.30, 128.68, 128.74, 141.52, 151.48 and 159.85 ppm. Compound, **4b** showed a base peak at *m/z*: 285.08 correspond to its molecular mass. All synthesized series of compounds **4**(**a-g**) showed similar and consistent pattern signals in their respective spectra and gave satisfactorily elemental analyses data with theoretically calculated values confirms their structures. Coupling constants (*J*) of 4-H and 5-H (*J* = 6.8-7.9 Hz) of compounds **4**(**a-g**) suggests that dipolar cycloaddition took place in *cis* fashion.

Since, isomeric compounds **5**(**a-g**) were obtained as minor products, the characterization was done only for few com-

pounds. Compound 1-[5-(furan-2-yl)-3-(4-methoxyphenyl)-4,5-dihydroisoxazol-4-yl]ethanone (**5b**) showed a singlets for three protons each at δ 2.320 and δ 3.840 ppm due to CH_3 and OCH_3 protons. The 4-H resonates as doublet at δ 2.690 (J = 7.0 Hz) ppm; while the 5-H appears as doublet at δ 4.980 (J = 7.5 Hz) ppm. Multiplets appear at δ 6.322-6.484 ppm for two and at δ 7.382-7.886 ppm for five protons were due toaromatic protons. The carbons of newly formed isoxazole ring 4-C, 5-C and 3-C coupled at δ 47.65, 65.30 and 152.84 ppm, respectively. Signals appeared at δ 27.98, 55.84 and 203.50 ppm were due to CH_3, OCH_3 and C=O carbons. Compound, **5b** showed a base peak at *m/z*: 285.04 correspond to its molecular mass. Similar and consistent pattern signals and satisfactorily elemental analyses data provides the structure proof of **5**(**a-g**).

The DPPH radical scavenging experiments were performed in triplicate; the results were expressed as a mean ± standard deviation (SD) and were summarized in Table-1.

TABLE-1
DPPH RADICAL SCAVENGING ACTIVITY OF THE SYNTHESIZED ISOXAZOLE DERIVATIVES **4**(**a-g**)

Compd.	Radical scavenging (%)*			
	25 µg/mL	50 µg/mL	75 µg/mL	100 µg/mL
4a	16.76±0.20	18.43±0.45	22.14±0.70	25.15±0.55
4b	20.10±0.44	25.18±0.51	29.10±0.66	39.20±0.75
4c	19.90±0.80	24.36±0.63	28.75±0.81	40.61±0.90
4d	20.55±0.76	26.40±0.54	32.84±0.45	39.20±0.38
4e	15.65±0.54	18.80±0.12	22.05±0.32	24.35±0.85
4f	14.11±0.32	16.20±0.80	20.10±0.50	22.26±0.70
4g	16.10±0.30	18.15±0.76	22.75±0.50	24.70±0.55
AA	15.08±0.89	17.87±0.89	21.98±0.31	24.25±0.22

*Values are mean±SD of three replicates; AA = Ascorbic acid (positive control)

Preliminary studies revealed that, the synthesized new pyrazole derivatives exhibited moderate to excellent DPPH radical scavenging abilities. Amongst the series, 1-[3-(4-chlorophenyl)-4-(furan-2-yl)-4,5-dihydroisoxazol-5-yl]ethanone (**4f**) showed excellent activity which is more than the standard ascorbic acid. Compounds 1-[4-(furan-2-yl)-3-phenyl-4,5-dihydroisoxazol-5-yl]ethanone (**4a**), 1-[3-(4-fluorophenyl)-4-(furan-2-yl)-4,5-dihydroisoxazol-5-yl]ethanone (**4e**) and 1-[4-(furan-2-yl)-3-(4-nitrophenyl)-4,5-dihydroisoxazol-5-yl]ethanone (**4g**) have showed good radical scavenging abilities. Compounds 1-[4-(furan-2-yl)-3-(4-methoxyphenyl)-4,5-dihydroisoxazol-5-yl]ethanone (**4b**), 1-[3-(3,4-dimethoxyphenyl)-4-(furan-2-yl)-4,5-dihydroisoxazol-5-yl]ethanone (**4c**) and 1-[4-(furan-2-yl)-3-(*p*-tolyl)-4,5-dihydroisoxazol-5-yl]ethanone (**4d**) showed moderate radical scavenging abilities. From the results, it was observed that the presence of an electron donating groups (OCH_3, CH_3) groups as aromatic ring substitutions retarded the DPPH radical scavenging ability; while electron withdrawing groups and electronegative atoms as substitutions enhanced the DPPH radical scavenging ability of the synthesized series of compounds.

The antimicrobial screening tests were performed in triplicate and the results were taken as a mean ± standard deviation (SD) and are summarized in Table-2.

Preliminary antimicrobial evaluation results showed that, amongst the series, compound **4f** showed excellent inhibition potential against all the tested organisms. Compounds **4a** and **4e** found good against *S. aureus, E. coli, A. niger* and *C. albicans:* moderately active against *E. coli* and *A. niger*; less active against *P. aeruginosa* and *A. flavus* organisms. Compounds **4b**, **4c** and **4d** found promisingly active against *S. aureus* moderately active against *E. coli* and *A. niger*; less active against *P. aeruginosa, A. flavus* and *C. albicans* organisms. Compound **4g** showed lesser inhibition ability against all the tested organisms, which might be attributed to the presence of strong electron withdrawing nitro substitution in the aromatic ring.

Conclusion

In the present study, the synthesis of isomeric mixture of isoxazoles *via* 1,3-dipolar cycloaddition reactions is reported. *In vitro* pharmacological evaluation results showed that, among the synthesized series, compounds **4a**, **4e**, **4g** and **4f** act as a potential DPPH radical scavengers, in particular compound **4f** which showed the ability which is greater than the standard ascorbic acid. The *in vitro* antimicrobial activity results of new isoxazolines reveals that the compounds **4a, 4e and 4f** may become a potential antifungal and antibacterial drug candidates.

ACKNOWLEDGEMENTS

The authors are grateful to IOE Instrumentation Facility, Vijnana Bhavana, University of Mysore, Mysuru, India for spectral analysis.

TABLE-2
MINIMUM INHIBITORY CONCENTRATION (MIC's) OF COMPOUNDS **4**(**a-g**) (µg/mL*)

Compound	*S. aureus*	*E. coli*	*P. aeruginosa*	*A. niger*	*A. flavus*	*C. albicans*
4a	25.0 ± 0.30	25.0 ± 0.25	50.0 ± 0.65	25.0 ± 0.50	75.0 ± 0.50	50.0 ± 0.30
4b	25.0 ± 0.35	50.0 ± 0.85	75.0 ± 0.65	50.0 ± 0.51	87.5 ± 0.86	87.5 ± 0.35
4c	25.0 ± 0.65	50.0 ± 0.51	75.0 ± 0.40	37.5 ± 0.65	75.0 ± 0.30	87.5 ± 0.51
4d	25.0 ± 0.30	50.0 ± 0.45	75.0 ± 0.76	50.0 ± 0.50	87.5 ± 0.70	87.5 ± 0.44
4e	25.0 ± 0.40	25.0 ± 0.40	75.0 ± 0.55	25.0 ± 0.30	87.5 ± 0.90	50.0 ± 0.75
4f	25.0 ± 0.40	25.0 ± 0.45	12.5 ± 0.30	25.0 ± 0.50	25.0 ± 0.60	50.0 ± 0.60
4g	75.0 ± 0.46	75.0 ± 0.30	50.0 ± 0.30	75.0 ± 0.50	75.0 ± 0.60	75.0 ± 0.50
Cipro[a]	25.0 ± 0.40	25.0 ± 0.60	12.5 ± 0.50	–	–	–
Nyst[b]	–	–	–	25.0 ± 0.45	25.0 ± 0.35	50.0 ± 0.90

*Values are mean ± SD of three replicates; [a]Ciproafloxacin-positive control against bacteria species; [b]Nystatin-positive control against fungi species.

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