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Synthesis of Isoxazolines: Small Molecules of Potent Antimicrobial Activity

Manjula N[1], Lokeshwari DM[1], Dileep Kumar A[1], Renuka N[2], Ajay Kumar K[1*]

[1]*Department of Chemistry, Yuvaraja's College, University of Mysore, India*
[2]*Department of Chemistry, GSSS Institute of Engineering and Technology for Women, Mysuru, India*

ABSTRACT

A simple procedure for the synthesis of isoxazole derivatives was developed. Cyclocondensation reaction of chalcones with hydroxylamine hydrochloride catalyzed by an acid produced isoxazoles in good yields. The synthesized new compounds were characterized by spectral studies and elemental analysis; before being were screened in vitro for their antimicrobial susceptibilities against different bacteria and fungi species.

Keywords: Antibacterial, Antifungal, Condensation, Inhibition, Isoxazoline

INTRODUCTION

Isoxazoles and their analogues are regarded as important molecules in organic synthesis. They serve as important synthons for the construction of various classes of bioactive molecules. Alkenes were regarded as useful precursors for designing the bioactive molecules such as pyrrolines [1] and isoxazolines [2]. The most convenient routes developed for the synthesis of isoxazole ring system has been executed in the literature via 1,3-dipolar cycloaddition reactions of alkenes and alkynes with nitrile oxides [3-5]. Nitrile oxides preferentially adds to olefinic C-C double or C-C triple bond than C-N triple bond of acrylonitrile to form isoxazolines [6,7]. The isoxazole derivatives have known to exhibit broad spectrum of biological applications, viz., antimicrobial [8], antioxidant [9], potent selective agonists at human cloned dopamine D4 receptors [10], γ-Aminobutyric Acid ($GABA_A$) antagonist [11], Cyclooxygenase (COX-2) inhibitory [12], antinociceptive [13], anticancer [14] activities. They serve as prodrug for the anti-antiarrhythmic agent [15].

In view of broad spectrum of synthetic and biological applications of isoxazole derivatives, and in search of new antimicrobial agents, we herein report the synthesis of series of new isoxazole derivatives and *in vitro* screening results of their antifungal, antibacterial activities.

MATERIALS AND METHODS

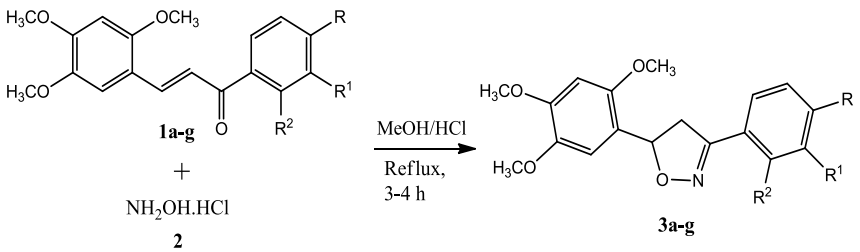
Melting points were determined by an open capillary tube method and are uncorrected. Purity of the compounds was checked on Thin Layer Chromatography (TLC) plates pre-coated with silica gel using solvent system ethyl acetate: Dichloromethane (1:4 v/v). The spots were visualized under UV light. Proton Nuclear Magnetic Resonance (^{1}H-NMR) and Carbon-13 Nuclear Magnetic Resonance (^{13}C-NMR) spectra were recorded on Agilent-NMR 400 MHz and 100 MHz spectrometer respectively. The solvent Deuterated Chloroform ($CDCl_3$) with Tetramethylsilane (TMS) as an internal standard was used to record the spectra. The chemical shifts are expressed in δ ppm. Mass spectra were obtained on Mass Lynx SCN781 spectrometer TOF mode. Elemental analysis was obtained on a Thermo Finnigan Flash EA 1112 CHN analyzer. Purification of compounds was done by column chromatography on silica gel (70-230 mesh Merck).

The synthesis of a series of isoxazolines, 3a-g involves; the cyclocondensation reaction of chalcones, 1a-f with hydroxylamine hydrochloride, 2 and few drops of concentrated hydrochloric acid in methyl alcohol under reflux conditions. The schematic diagram for the synthesis of isoxazolines is outlined in Figure 1.

The required intermediate chalcones, 1a-g were obtained according to our reported procedure by condensation of 2,4,5-trimethoxybenzaldehyde and acetophenones, in the presence of potassium hydroxide in 95% ethyl alcohol at room temperature [16-18].

General procedure for the synthesis of isoxazoles, 3a-g

To a stirred solution of chalcones, 1a-g (0.01 mol) and hydroxylamine hydrochloride, 2 (0.01 mol) in methyl alcohol (15 ml), Conc. hydrochloric acid (7-8 drops) were added. The mixture was refluxed for 3-4 h, the progress of the reaction was monitored by TLC. After completion, the reaction mixture was poured in to ice cold water; solids separated were filtered, washed with ice cold water and dried. The products were purified column chromatography using silica gel (60-120 mesh) and Ethyl acetate: Hexane (1:4 v/v) as mobile phase.



3 a) R=H, R^1=H, R^2=H; b) R=F, R^1=H, R^2=H; c) R=Cl, R^1=H, R^2=H;
d) R=OCH_3, R^1=H, R^2=H; e) R=H, R^1=OCH_3, R^2=H; f) R=H, R^1=H, R^2=OCH_3;
g) R=R^1=OCH_2O, R^2=H.

Figure 1: Schematic diagram for the synthesis of isoxazolines, 3a-g

3-Phenyl-5-(2,4,5-trimethoxyphenyl)-4,5-dihydroisoxazole, 3a

Obtained from 1-phenyl-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one, 1a (10 mmol) and hydroxylamine hydrochloride, 2 (10 mmol) in 66% yield, m.p. 110-112°C. ^{1}H-NMR: δ=3.336-3.460 (dd, 1H, J=7.0, 13.6Hz, C_4-H_a), 3.702-3.789 (dd, 1H, J=8.0, 14.5Hz, C_4-H_b), 3.852 (s, 9H, OCH_3), 5.778-5.834 (dd, 1H, J=6.5, 12.3Hz, C_5-H), 6.981-7.665 (m, 7H, Ar–H); ^{13}C-NMR: δ=42.45 (1C, C-4), 55.45 (3C), 74.46 (1C, C-5), 101.56 (1C), 111.80 (1C), 121.08 (1C), 128.32 (2C), 129.32 (2C), 130.65 (1C), 131.20 (1C), 144.96 (1C), 147.33 (1C), 150.40 (1C), 155.10 (1C, C-3). MS *m/z*: 314 (MH^+, 100); Anal. Calcd. for $C_{18}H_{19}NO_4$ (%): C, 68.99; H, 6.11; N, 4.47; Found: C, 68.79; H, 6.00; N, 4.30.

3-(4-Fluorophenyl)-5-(2,4,5-trimethoxyphenyl)-4,5-dihydroisoxazole, 3b

Obtained from 1-(4-fluorophenyl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one, 1b (10 mmol) and hydroxylamine hydrochloride, 2 (10 mmol) in 80% yield. ^{1}H-NMR: δ=3.344-3.489 (dd, 1H, J=7.3, 13.0Hz, C_4-H_a), 3.712-3.770 (dd, 1H, J=6.0, 11.5Hz, C_4-H_b), 3.855 (s, 9H, OCH_3), 5.770-5.839 (dd, 1H, J=6.1, 13.6Hz, C_5-H), 6.752-6.920 (m, 2H, Ar–H); 7.351-7.524 (dd, 2H, Ar–H); 7.750-7.824 (dd, 2H, Ar–H); ^{13}C-NMR: δ=42.40 (1C, C-4), 55.44 (3C), 74.60 (1C, C-5), 101.26 (1C), 110.86 (1C), 115.30 (2C), 123.08 (1C), 126.60 (1C), 129.40 (2C), 144.80 (1C), 147.56 (1C), 150.44 (1C), 155.36 (1C, C-3), 164.21 (1C). MS *m/z*: 332 (MH^+, 100); Anal. Calcd. for $C_{18}H_{18}FNO_4$ (%): C, 65.25; H, 5.48; N, 4.23; Found: C, 65.18; H, 5.31; N, 4.18.

3-(4-Chlorophenyl)-5-(2,4,5-trimethoxyphenyl)-4,5-dihydroisoxazole, 3c

Obtained from 1-(4-chlorophenyl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one, 1c (10 mmol) and hydroxylamine hydrochloride, 2 (10 mmol) in 69% yield. ^{1}H=NMR: δ=3.348-3.487 (dd, 1H, J=6.5, 11.7Hz, C_4-H_a), 3.720-3.771 (dd, 1H, J=6.9, 13.1Hz, C_4-H_b), 3.848 (s, 9H, OCH_3), 5.766-5.840 (dd, 1H, J=8.1, 15.4Hz, C_5-H), 6.780-6.930 (m, 2H, Ar–H); 7.356-7.530 (dd, 2H, Ar-H); 7.755-7.825 (dd, 2H, Ar–H); ^{13}C-NMR: δ=41.90 (1C, C-4), 55.56 (3C), 74.87 (1C, C-5), 101.10 (1C), 110.60 (1C), 123.14 (1C), 126.60 (1C), 128.10 (2C), 128.36 (1C), 129.02 (2C), 144.66 (1C), 147.50 (1C), 150.22 (1C), 155.40 (1C, C-3). MS *m/z*: 349 (M^+, ^{37}Cl, 34), 347 (M^+, ^{35}Cl, 100); Anal. Calcd. for $C_{18}H_{18}ClNO_4$ (%): C, 62.16; H, 5.22; N, 4.0; Found: C, 62.10; H, 5.12; N, 3.90.

3-(4-Methoxyphenyl)-5-(2,4,5-trimethoxyphenyl)-4,5-dihydroisoxazole, 3d

Obtained from 1-(4-methoxyphenyl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one, 1d (10 mmol) and hydroxylamine hydrochloride, 2 (10 mmol) in 72% yield; ^{1}H-NMR: δ=3.332-3.467 (dd, 1H, J=8.1, 15.5Hz, C_4-H_a), 3.710-3.760 (dd, 1H, J=6.9, 14.2Hz, C_4-H_b), 3.856 (s, 12H, OCH_3), 5.756-5.820 (dd, 1H, J=7.1, 12.3Hz, C_5-H), 6.758-6.916 (m, 2H, Ar–H); 7.380-7.510 (dd, 2H, Ar–H); 7.76-7.820 (dd, 2H, Ar–H); ^{13}C-NMR: δ=42.55 (1C, C-4), 55.62 (4C), 74.68 (1C, C-5), 101.21 (1C), 110.76 (1C), 115.32 (2C), 122.65 (1C), 123.05 (1C), 129.10 (2C), 144.83 (1C), 147.46 (1C), 150.39 (1C), 155.20 (1C, C-3), 162.20 (1C). MS *m/z*: 344 (MH^+, 100); Anal. Calcd. for $C_{19}H_{21}NO_5$ (%): C, 66.46; H, 6.16; N, 4.08; Found: C, 66.36; H, 6.02; N, 4.01.

3-(3-Methoxyphenyl)-5-(2,4,5-trimethoxyphenyl)-4,5-dihydroisoxazole, 3e

Obtained from 1-(3-methoxyphenyl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one, 1e (10 mmol) and hydroxylamine hydrochloride, 2 (10 mmol) in 80% yield. ^{1}H-NMR: δ=3.330-3.462 (dd, 1H, J=8.5, 16.2Hz, C_4-H_a), 3.718-3.756 (dd, 1H, J=6.8, 13.9Hz, C_4-H_b), 3.852 (s, 12H, OCH_3), 5.755-5.821 (dd, 1H, J=7.3, 12.7Hz, C_5-H), 6.790–7.620 (m, 6H, Ar–H); ^{13}C NMR: δ=42.50 (1C, C-4), 55.65 (4C), 74.64 (1C, C-5), 101.40 (1C), 110.55 (1C), 115.30 (2C), 122.45 (1C), 123.22 (1C), 129.16 (2C), 144.87 (1C), 147.41 (1C), 150.42 (1C), 155.23 (1C, C-3), 162.25 (1C). MS *m/z*: 344 (MH^+, 100); Anal. Calcd. for $C_{19}H_{21}NO_5$ (%): C, 66.46; H, 6.16; N, 4.08; Found: C, 66.32; H, 6.04; N, 4.00.

3-(2-Methoxyphenyl)-5-(2,4,5-trimethoxyphenyl)-4,5-dihydroisoxazole, 3f

Obtained from 1-(2-methoxyphenyl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one, 1f (10 mmol) and hydroxylamine hydrochloride, 2 (10 mmol) in 75% yield. ^{1}H-NMR: δ=3.325-3.460 (dd, 1H, J=8.0, 15.0Hz, C_4-H_a), 3.712-3.755 (dd, 1H, J=6.5, 14.2Hz, C_4-H_b), 3.855 (s, 12H, OCH_3), 5.750-5.827 (dd, 1H, J=7.0, 12.9Hz, C_5-H), 6.795-7.626 (m, 6H, Ar–H); ^{13}C-NMR: δ=42.50 (1C, C-4), 55.60 (4C), 74.64 (1C, C-5), 101.43 (1C), 110.56 (1C), 115.37 (2C), 122.63 (1C), 123.09 (1C), 129.13 (2C), 144.84 (1C), 147.34 (1C), 150.54 (1C), 155.66 (1C, C-3), 162.27 (1C). MS *m/z*: 344 (MH^+, 100); Anal. Calcd. for $C_{19}H_{21}NO_5$ (%): C, 66.46; H, 6.16; N, 4.08; Found: C, 66.36; H, 6.02; N, 4.01.

3-(Benzo[d][1,3]dioxol-5-yl)-5-(2,4,5-trimethoxyphenyl)-4,5-dihydroisoxazole, 3g

Obtained from 1-(benzo[d][1,3]dioxol-5-yl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one, 1g (10 mmol) and hydroxylamine hydrochloride, 2 (10 mmol) in 81% yield. ^{1}H-NMR: δ=3.350-3.476 (dd, 1H, J=9.1, 16.2Hz, C_4-H_a), 3.720-3.776 (dd, 1H, J=7.0, 12.5Hz, C_4-H_b), 3.860 (s, 9H, OCH_3), 5.776-5.843 (dd, 1H, J=6.5, 13.2Hz, C_5-H), 6.021 (s, 2H, OCH_2O); 6.951-7.422 (m, 5H, Ar–H); ^{13}C-NMR: δ=41.90 (1C, C-4), 55.54 (3C), 74.65 (1C, C-5), 101.10 (1C), 102.76 (1C), 110.81 (1C), 111.30 (1C), 114.56 (1C), 120.65 (1C), 123.25 (1C), 126.38 (1C), 144.68 (1C), 147.42 (1C), 148.10 (1C), 150.38 (1C), 151.33 (1C), 155.60 (1C, C-3). MS *m/z*: 359 (MH^+, 100); Anal. Calcd. for $C_{19}H_{19}NO_6$ (%): C, 63.86; H, 5.36; N, 3.92; Found: C, 63.76; H, 5.25; N, 3.78.

RESULTS AND DISCUSSION

Structure proof of compounds, 3a-g were obtained by ^{1}H-NMR, ^{13}C-NMR, Mass spectral studies and elemental analysis. Amongst the series, 3-(4-chlorophenyl)-5-(2,4,5-trimethoxyphenyl)-4,5-dihydroisoxazole, 3c was considered as a representative compound for NMR analysis. In its ^{1}H-NMR spectrum, two methylene protons designated as C_4-H_a and C_4-H_b of the newly formed isoxazole ring exhibit typical ABX spin and are diastereotopic. The C_4-H_a and C_4-H_b protons appeared as a doublet of doublets. The doublet of doublet for C_4-H_a appeared at δ=3.348-3.487 (J=6.5, 11.7Hz) ppm; doublet of doublet for C_4-H_b appeared at δ=3.720-3.771 (J=6.9, 13.1Hz) ppm. C_5-H couples not only with C_4-H_a but also with C_4-H_b and appears as doublet of doublet at δ=5.766-5.840 (J=8.1, 15.4Hz) ppm instead of a triplet. Amongst the C_4-H_a, C_4-H_b and C_5-H protons, C_5-H is the most deshielded due to its close proximity to benzene ring and electronegative oxygen atom. A signal appearing as singlet for nine protons at δ=3.848 ppm was assigned to OCH_3 protons. A collection of signals observed for two protons each at δ=6.780-6.930 ppm, δ=7.356-7.530 ppm and δ=7.755-7.825 ppm were assigned to aromatic protons.

In ^{13}C-NMR spectrum, compound 3c showed a signal at δ=41.90, 74.87 and 155.40 ppm due to C-4, C-5 and C-3 carbons of the isoxazole ring. A signal appeared for three carbons at δ=55.56 ppm was assigned to three OCH_3 carbons. An array of signals one carbon each appeared at δ=101.10, 110.60, 123.14, 126.60, 128.36, 144.66), 147.50, 150.22 ppm and for two carbons each at δ=128.10, 129.02 ppm were ambiguously assigned to aromatic carbons. Compound 3c showed molecular ion peak at m/z 349 with a relative abundance of 34% corresponding to corresponding to its molecular mass with ^{37}Cl isotope, and a base peak at m/z 347 corresponds to ^{35}Cl isotope. Further, satisfactory elemental analysis data obtained for the compound were in good agreement with theoretically calculated values. Compounds 3a-g shows similar and consistent pattern signals in their ^{1}H-NMR, ^{13}C-NMR and Mass spectra, which strongly supports the structure proof for the synthesized compounds.

Antimicrobial activity

Antimicrobial studies of synthesized compounds 3a-g were assessed by Minimum Inhibitory Concentration (MIC) by serial dilution method [19]. The compounds were screened for their antimicrobial activities against Gram-negative bacteria (*Escherichia coli*), Gram-positive bacteria (*Staphylococcus aureus*), fungi species (*Aspergillus nigar*) and (*Aspergillus flavus*). The experiments were carried out in triplicate; the results were expressed as a mean of three determinations (n=3). The antibiotics ciprofloxacin and nystatin were used as standard drugs for antibacterial and antifungal studies respectively. The results of MIC's were depicted in Figure 2.

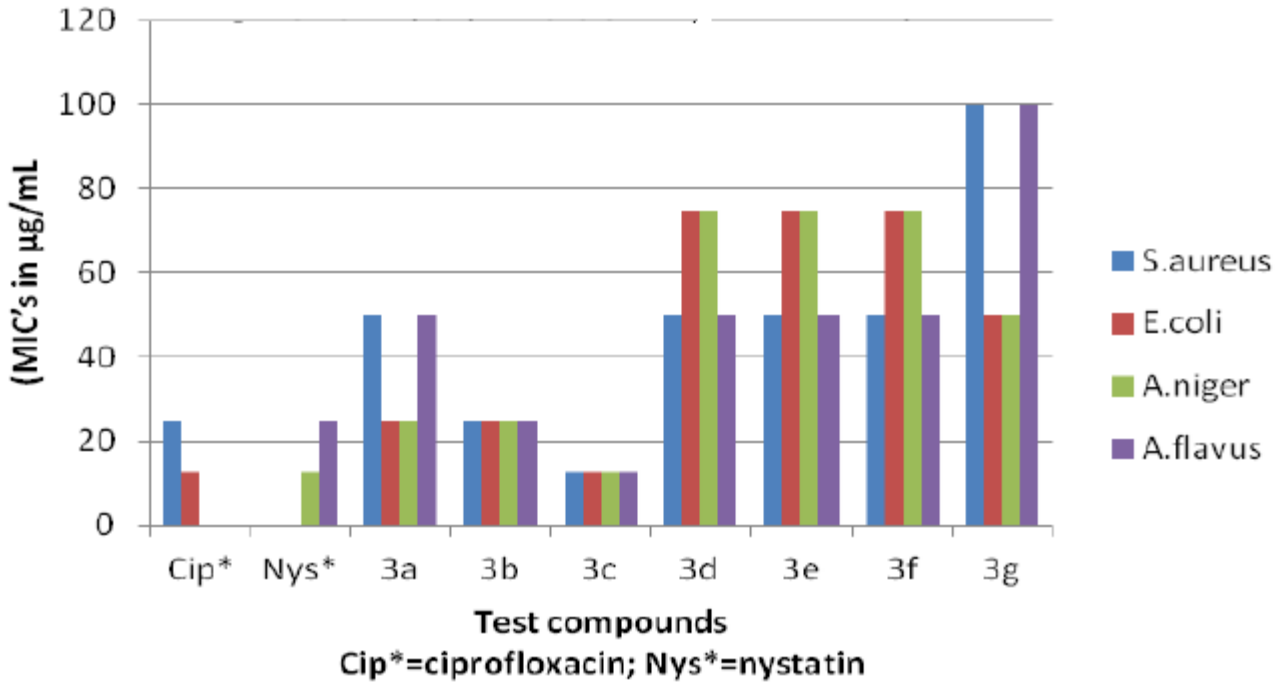


Figure 2: Minimum Inhibitory Concentration (MIC) of the compounds, 3a-g: Values are expressed as mean of three determinations

The synthesized series of isoxazoles, 3a-g exerted a wide range of *in vitro* antimicrobial activities against the tested organisms. Preliminary studies reveal that, amongst the series, compound 3c having chloro substitution showed an excellent inhibition potential against all the tested species. Compound 3b with fluoro substituted aromatic ring found promisingly active against *E. coli* and *A. flavus* and moderate against *S. aureus* and *A. niger* species. Compounds 3a, 3d, 3e and 3f showed moderate inhibitory effect against *S. aureus* and *A. flavus* while, 3g found moderately active against *E. coli* and *A. niger* organisms. Compounds 3d, 3e and 3f showed poorer inhibitory effect against *E. coli* and *A. niger* while, 3g found less active against *S. aureus* and *A. flavus* organisms.

CONCLUSION

The simple procedure for the synthesis of isoxazilines was described. Preliminary investigations on *in vitro* antimicrobial activity studies on the synthesized isoxazoles validate the significance of the study. Amongst the series, compound 3-(4-chlorophenyl)-5-(2,4,5-trimethoxyphenyl)-4,5-dihydroisoxazole, 3c demonstrated excellent antimicrobial activity against the tested microorganisms and can be used as potent antimicrobial agent.

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