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SYNTHESIS, CHARACTRIZATION AND ANTIMICROBIAL ACTIVITY OF 3,5-DIARYL-4,5-DIHYDRO-2-ISOXAZOLINE DERIVATIVES: STRUCTURE-ACTIVITY AND RELATIONSHIP

Ravi R. Vidule[a] and Madhav M. Kendre[a]*

[a]Department of Chemistry, Shri Sant Gadge Maharaj Mahavidylaya Loha, Nanded-431608, India.

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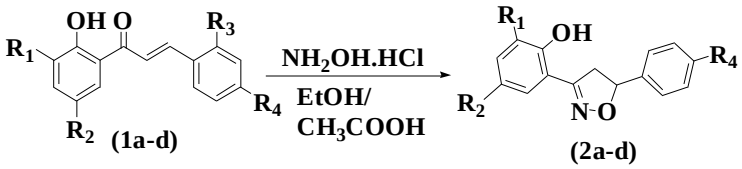
***Corresponding Author**
Madhav M. Kendrea
Department of Chemistry,
Shri Sant Gadge Maharaj
Mahavidylaya Loha,
Nanded-431608, India.

ABSTRACT

Isoxazoline is the five member heterocyclic compound containing nitrogen and oxygen are found to be new sources for the synthesis of biologically active derivatives. Isoxazolines are the azole in which an oxygen atom is present next to nitrogen. These are found in a large number of natural products and in biologically active molecules. The derivatives of isoxazolineconstitutes different classes of heterocycles having diverse applications, including agrochemicals as fungicides, insecticides, drugs, dyes, rubber chemicals, flavoring agents and in materials science. These isoxazolines represent an important heterocyclic system commonly found in natural products and bioactive compounds. A novel series of 3,5-diaryl-4,5-dihydro-2-isoxazolines (**2a-d**) from (*E*)-1-(2-hydroxy-3,5-substituted-phenyl)-3-(2/4-ethoxy-phenyl)-prop-2-en-1-one (**1a-d**) in ethanol by the addition of hydroxylamine hydrochloride and sodium acetate in the acidic medium by refluxing in oil-bath. The synthesized derivatives were characterized by FT-IR, ^{1}H NMR, ^{13}C NMR, LCMS and elemental analysis. All these synthesized derivatives screened for antibacterial and antifungal activities and are showing potent antimicrobial activities.

KEYWORDS: Chalcones, Isoxazoline, hydroxylamine hydrochloride, sodium acetate, Antimicrobial activities.



R_1 = Br, Br, I, I; R_2 = Cl, Me, Cl, I; R_4 = OEt

INTRODUCTION

Isoxazoline is the five member heterocyclic compound containing nitrogen and oxygen are found to be new sources for the synthesis of biologically active derivatives. Isoxazolines are the azole in which an oxygen atom is present next to nitrogen. These are found in a large number of natural products and in biologically active molecules.[1] The derivatives of isoxazolineconstitutes different classes of heterocycles having diverse applications, including agrochemicals as fungicides, insecticides, drugs, dyes, rubber chemicals, flavoring agents and in materials science. These isoxazolines represent an important heterocyclic system commonly found in natural products, bioactive compounds, and chiral ligands.[2–13]

Moreover isoxazolines are important synthetic building blocks towards synthesis of bio-active molecules, including antiviral,[14] anti-inflammatory,[15,16] and anticancer activities.[17-21] These isoxazolines are particularly relevant as they form the core structure of many currently used therapeutic agents, veterinary products and agrochemicals.[22] The continued interest in isoxazolines is not limited to their biological properties but equally their routine use for the reductive ring opening to provide 1,3-dicarbonyl compounds.[23]

Generally, these heterocycles are assembled via a [3+2] dipolar cycloaddition of nitrile oxides, themselves typically prepared from sometimes unstable hydroxymoyl chlorides,[24] or by the dehydration of nitroalkanes.[25-26] The relevance of isoxazolines in organic and medicinal chemistry, the structures of boilogically active[27] isoxazolines are shown in **Fig** (1).

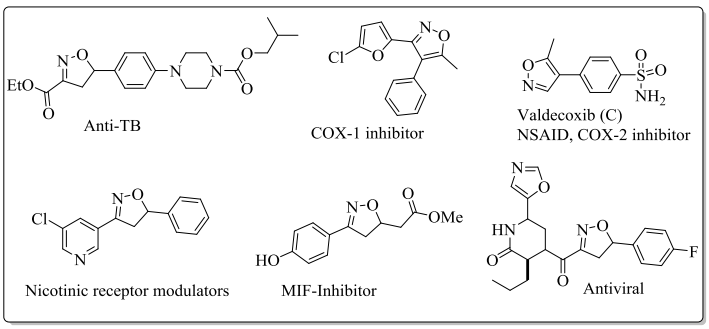


Figure 1: Examples of some bioactive isoxazolines.

Experimental

Chemicals and reagents

Substituted derivatives of chalcone, hydroxylamine hydrochloride, acetic acid, and ethanol.

Experimental procedure: Synthesis of 3,5-diaryl-4,5-dihydro-2-isoxazolines (2a-d)

The 3,5-diaryl-4,5-dihydro-2-isoxazolines (**2a-d**) were synthesized by the condensation of substituted derivatives of (*E*)-1-(2-hydroxy-3,5-substituted-phenyl)-3-(2/4-ethoxy-phenyl)-prop-2-en-1-one (**1a-d**)(1 mmol) with hydroxylamine hydrochloride (1.5 mmol) in ethanol in an acidic medium using acetic acid. The reaction mixture was refluxed for 2.5 hours by using ethanol as a solvent in oil-bath. The completion of reaction was monitored by TLC.

The reaction mixture was cooled to room temperature and poured into ice cold water. The separated solid was filtered, washed several times with cold water and cold ethanol water mixture to remove impurities, dried and crystallized from ethanol to afford 3,5-diaryl-4,5-dihydro-2-isoxazolines (**2a-d**). The structures of synthesized compounds were confirmed by IR, ^{1}H NMR and Mass spectral analysis.

Methods of synthesis of Isoxazolines

Till date numbers of methods are present in literature for the synthesis of isoxazoline moieties from the different derivatives chalcone with hydroxylamine hydrochloride by conventional and non conventional methods.[28-34]

Table 1: Physical data of synthesizedisoxazolinederivatives (2a-d).

Sr. No.	Entry	Molecular formula	Yield %	Melting Point ^{0}C
1	**2a**	$C_{17}H_{15}BrClNO_3$	77	143
2	**2b**	$C_{18}H_{18}BrNO_3$	81	153
3	**2c**	$C_{17}H_{15}ClINO_3$	79	164
4	**2d**	$C_{17}H_{15}I_2NO_3$	76	156

General procedure for the synthesis of 3,5-diaryl-4,5-dihydro-2-isoxazolines (2a-d)

A mixture of substituted derivatives of (*E)*-1-(2-hydroxy-3,5-substituted phenyl)-3-(4/2-ethoxyphenyl)-prop-2-en-1-one (**1a-d**) (1 mmol) in 15ml ethyl alcohol then added hydroxylamine hydrochloride (1.5 mmol) and sodium acetate (1 mmol). This reaction mixture was acidified by the addition of few drops of acetic acid and refluxed in oil-bath for 2.5 hours. The completion of reaction was checked with TLC, upon completion of reaction

the content was cooled to room temperature and then all the content was poured into ice cold water.

The separated solid was filtered, washed with cold water and cooled ethanol water mixture several times to remove the impurities, dried then obtained solid was recrystallized by using ethanol. We have synthesized new series of3,5-diaryl-4,5-dihydro-2-isoxazolines (**2a-d**), the structures of the selected synthesized compounds of isoxazoline were confirmed by IR, ^{1}H NMR, LCMS, and elemental analysis. The newly synthesized compounds were subjected to antimicrobial activity.

3.4 DISCUSSION OF SPECTRA

The IR spectra of the synthesized compounds recorded on a Fourier Transform IR spectrometer (model Shimadzu 8700) in the range of 400-4000 cm^{-1} using KBr pellets.

^{1}H NMR spectra recorded on Bruker 400 MHz NMR spectrometer using $CDCl_3$ and the chemical shifts (δ) reported are in ppm downfield using tetramethylsilane (TMS) as internal reference. The NMR data is reported as follows: chemical shift, multiplicity (s = single, d = doublet, t = triplet, q = quartet, m = multiplet. The coupling constant (J) is given in Hz.

The mass spectra (MS) were recorded on EI-SHIMADZU-GC-MS mass spectrometer. The purity of all new derivatives was confirmed by elemental analysis (CHN) using thermofinnigan flash EA 1112 Thermo Finnigan with more than 95%. The melting points were determined open capillary method and are uncorrected.

DISCUSSION OF SPECTRA

Spectral interpretation of 3,5-diaryl-4,5-dihydro-2-isoxazolines (2a-d)

IR Spectra of 3,5-diaryl-4,5-dihydro-2-isoxazolines

The cyclization of (*E)*-1-(2-hydroxy-3,5-substitutedphenyl)-3-(4/2-ethoxyphenyl)-prop-2-en-1-one (**1a-d**)to 3,5-diaryl-4,5-dihydro-2-isoxazolines (**2a-d**) was further supported by IR spectral data, picks due to (>C=O) stretching observed at 1616-1691 cm^{-1} due to (-N=C<) stretching. The band in the range 1390-1396 cm^{-1} indicates the presence of (-OCH_2CH_3) group. The absorption band at 3254-3338 cm^{-1} is due to –OH stretching. The bands 785 cm^{-1} due to >C-Cl stretching, 601 cm^{-1} due to >C-Br appear whenever these groups were present in the respective compound. All these observations are in agreement with those observed earlier.[61-65]

^{1}H NMR spectra

^{1}H-NMR spectrum of compound displayed three characteristic signals due to diasterotopic protons H_A, H_B, H_x, pattern of isoxazoline ring were seen as doublet of doublet. The H_A proton which is cis to Hx resonates upfield at δ 2.88-2.50 ppm as doublet of doublets, while the H_B proton which is trans to Hx resonates downfield δ 3.33–3.27 ppm. The Hx proton which is vicinal to two methylene protons (H_A and H_B) is also observed as double doublet at δ value in between 5.30-5.20 ppm (dd, J = 9.42 and Hz). The compilation of signals of multiplet of aromatic hydrogen appeared in the expected chemical shift and integral values in between region δ 7.85-6.95 ppm. Theexchangeable proton of ortho-hydroxyl group appeared as a ^{1}H singlet in the region in the range δ11.72-11.47 ppm.[61-65]

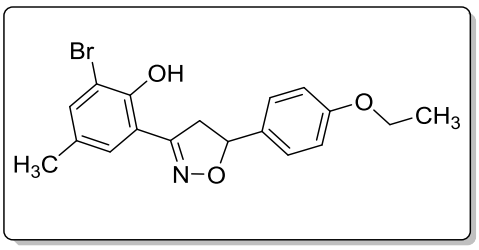
While the protons belonging to the aromatic ring and aliphatic protons were observed at expected regions. The desire peaks were observed as per the earlier findings of literature values. The triplet for -CH_3 of ethoxy group appeared in the region δ1.35-1.31 ppm and the quintet of -CH_2 for ethoxy group appeared in the region δ 4.07-4.01 ppm, and the singlet for the Ar-CH_3 appeared in the region δ 2.25 ppm respectively.

Mass spectra

The mass spectra of synthesized products of isoxazolines are also in agreement with their molecular formulae weights.

Spectral data of synthesized compounds

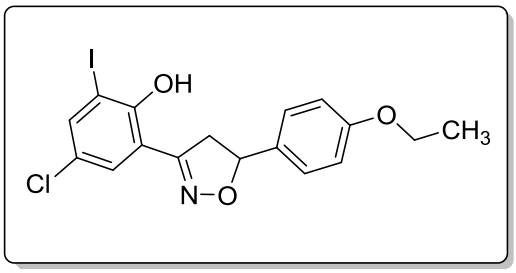
1. **3-(3-Bromo-2-hydroxy-4-methylphenyl)-5-(4-ethoxyphenyl)-4,5-dihydro-2-isoxazoline (2b)**



IR (KBR cm^{-1}):- 3338, 1691, 1598, 1396, 601, cm^{-1}; **^{1}H NMR (400 MHz, $CDCl_3$, ppm) :-** δ 11.47 (s, 1H, OH), 6.95-7.60 (m, 11H, Ar-H), 5.20-5.24 (t, 1H, H_X), 4.01 – 4.06 (q, 2H, -OCH_2), 3.27-3.32 (dd, 1H, H_B), 2.81-2.88 (dd, 1H, H_A), 2.25(s, 3H, -CH_3), 1.31-1.35 (t, 3H, -CH_3 of ethoxygp); **^{13}C-NMR ($CDCl_3$):-** δ 168.4, 158.7, 158.5, 137.3, 134.2, 133.0, 127.2, 127.1, 121.4, 121.2, 115.6, 114.4, 114.3, 84.1, 64.0, 42.5, 20.2, 14.8; **LCMS (m/z):-** 376;

CHN analysis:- Calculated for $C_{18}H_{18}BrNO_3$: C, 57.46; H, 4.82; N, 3.72; found C, 57.32; H, 4.86; N, 3.78.

2. **3-(5-chloro-2-hydroxy-3-iodophenyl)-5-(4-ethoxyphenyl)-4,5-dihydro- 2-isoxazoline (2c)**



IR (KBR cm^{-1}):- 3254, 1616, 1390, 785, 431 cm^{-1}; **^{1}H NMR (400 MHz, $CDCl_3$, ppm):-** ***δ***11.72 (s, 1H, OH), 6.96 - 7.85 (m, 6H, Ar-H), 5.26-5.30 (t, 1H, H_X), 4.02 – 4.07 (q, 2H, -OCH_2), 3.29-3.33 (dd, 1H, H_B), 2.50-2.87 (dd, 1H, H_A), 1.32-1.35(t, 3H, -CH_3 of ethoxygp); **^{13}C-NMR ($CDCl_3$):-** *δ* 158.7, 158.6, 156.7, 141.9, 134.3, 129.0, 128.7, 126.2, 126.1, 121.5, 114.4, 114.3, 89.3, 84.1, 64.0, 42.3, 14.8; **LCMS (m/z):-** 443; **CHN analysis:-** Calculated for$C_{17}H_{15}ClINO_3$: C, 46.02; H, 3.41; N, 3.16; Found C, 46.15; H, 3.38; N, 3.21.

1.3 Antimicrobial assay of the compounds

As in the past and current reviews of literatures the synthetic compounds like chalcones,[35-38] pyrazolines,[39,40] and isoxazolines,[41,42] are acting as potentially useful bactericidal and fungicidal agents. The selected compounds from the newly synthesized compounds of isxazolinederivatives (**2a-d**) were screened for the antibacterial activity gram(+ve) bacteria *Staphylococcus aureus*, *Bacillus subtilis* and against gram(-ve) bacteria *Escherichia coli* (*E. coli*), *Salmonella typhi* by using Agar cup method the assay was conducted by using the Nutrient Agar, here we have used Penicillin as standard drug for reference.[20,21]

The antifungal activities of the selected synthesized compounds carried out by using *Aspergillus Niger*, Penicillium*chrysogenum*, *FusariumMoneliforme* and *Aspergillusflavus*, by using Poison Plate method[43,44] the potato dextrose agar (PDA) plates were prepared by using pour plate technique for each extract and here we have used using Griseofulvin as standard drug for reference.

Table 2: Antibacterial Screening of synthesized isoxazoline derivatives(2a-d).

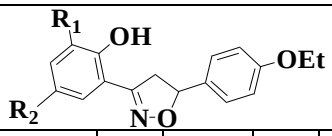
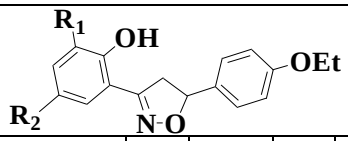
					Antibacterial zone of inhibition (in mm)			
					Microorganism			
Entry	**R_1**	**R_2**	**R_3**	**R_4**	***E. Coli***	***S. typhi***	***S. aureus***	***B.Substilis***
2a	Br	Cl	H	OEt	15	-ve	14	17
2b	Br	CH_3	H	OEt	13	-ve	16	19
2c	I	Cl	H	OEt	-ve	-ve	15	17
2d	I	I	H	OEt	-ve	-ve	16	13
DMSO	**……..**				-ve	-ve	-ve	-ve
Penicillin	**……**				11	24	36	30
-ve no antibacterial activity								

Table 3: Antifungal Screening of synthesized isoxazoline derivatives (2a-d).

					Microorganism			
Entry	**R_1**	**R_2**	**R_3**	**R_4**	***Asp Niger***	***Pen. Chrysogenum***	***Fusarium Moneliforme***	***Asppergillusflavus***
2a	Br	Cl	H	OEt	+ve	-ve	-ve	-ve
2b	Br	CH_3	H	OEt	+ve	-ve	-ve	RG
2c	I	Cl	H	OEt	-ve	-ve	-ve	RG
2d	I	I	H	OEt	+ve	-ve	-ve	+ve
DMSO	**……..**				+ve	+ve	+ve	+ve
Griseofulvin	**……**				-ve	-ve	-ve	-ve
+ve Growth (Antifungal activity absent), **-ve** No Growth (more than 90% reduction in growth Antifungal activity present), **RG** – Reduced Growth (more than 50% and less than 90% growth)								

RESULT AND DISCUSSION

The antimicrobial activity of selected synthesized derivatives of isoxazoline was obtained by previously reported biological methods. The selected synthesized compounds (**2a-d**) were screened for Antimicrobial activity.

All the novel derivatives of isoxazolinecompound were screened for the antimicrobial activites. Among the screened compounds all the derivatives **a, b, c** and **d** showed significant antibacterial activity against *Staphylococcus aureus*, *Bacillus Substilis*and moderate activity against *E. Coli* but for the *Salmonella typhi*the activity absent for the all derivatives as compared with Penicillin the data presented in **Table No**. 3 and 4.

The antifungal activities were tested for the chalcones**a, b, c** and **d,** against the four pathogens *Aspergillusniger*, *PenicilliuChrysogenum*, *FusariumMoneliforme*, *Aspergillusflavus* the

compounds from **Table No**.4 the derivatives **b** and **c** are showing more than 90% antifungal activity for *PenicilliuChrysogenum*, *FusariumMoneliforme*.

The compounds substituted with electron donating ethoxy group and electron donating halogens on the aromatic nucleus exhibiting best antimicrobial activities.

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