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Synthesis, Biological Evaluation of Novel 3,5-diarylsubstituted isoxazolines as Potential Antimicrobial and Cytotoxic Agents

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ABSTRACT

Novel 3,5-diarylsubstituted isoxazolines (IS 01-10) were synthesized by the condensation of 1-[4-(1H-pyrrol-1-yl)phenyl]ethanone chalcones with hydroxylamine hydrochloride and characterized using FT-IR, Mass, NMR and elemental analysis. These compounds were evaluated for their antibacterial activity against four micro-organisms, namely *Staphylococcus aureus* (MTCC 96), *Bacillus subtilis* (MTCC 441), *Escherichia coli* (MTCC 443), *Pseudomonas vulgaris* (MTCC 2421). The synthesized compounds also evaluated for cytotoxic activity against human cancer cell lines. The human tumour cell line panel constituted three cancer cell lines including breast (MCF-7), colon (HT-29) and prostate (DU-145). Among all, compound IS-06 showed greater inhibitory activity against all tested organisms employedwith zones of inhibition of 35 to 22 mm at a concentration of 150µg/ml. Compounds IS-04 & IS-07 have been found as the next in the order of its antimicrobial potency. In cytotoxic studies, compound IS-02found to the potent one with IC_{50} values of 18 µg/mL, 23 µg/mL and 20 µg/mL against MCF-7, HT-29& DU-105 cell lines respectively.Compound IS-01 was next in order with IC_{50} values of 24 µg/mL, 38 µg/mL and 28 µg/mL against MCF-7, HT-29& DU-105 cell lines respectively.

KEY WORDS: Chalcone, Isoxazoline, Condensation, Antimicrobial, Cytotoxic.

1. INTRODUCTION

Among five membered heterocycles, isoxazoline represents a class of compounds of great importance in biological chemistry. For instance, isoxazoline possess biological activities like insecticidal, antibacterial, antibiotic, antitumour, antifungal, anti-inflammatory and analgesic (Huisgen, 1963; Velikorodov and Sukhenko, 2003; Sharma and Sharma, 2010; Ankhiwala and Hathi, 1995; Sudhir, 2012; Kedar, 1997; Tangallapally, 2007; Habeeb, 2001). Isoxazoline also serves as anti-influenza virus activity (Kai, 2001), inhibition of human leukocyte elastase and cathepsin G (Groutas, 1992). In fact, valdecoxib an isoxazoline derivative is now widely used in the market as anti-inflammatory drug (Dannhardt, 2000). Benzofuran isoxazolines serves as protein tyrosine phosphatase 1B inhibitors (Ahmad, 2006). Isoxazoline derivatives also have been found to exhibit anti tubercular activity (Kachhadia, 2004). Keeping in view the biological and medicinal importance of chalcones and isoxazolines, we have synthesized some isoxazolines starting from substituted 1-[4-(1H-pyrrol-1-yl)phenyl] ethanone chalcones and hydroxylamine hydrochloride.

2. MATERIALS AND METHODS

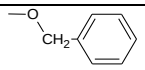
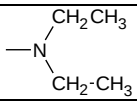
Melting points were determined using Boethius Apparatus by capillary method and are uncorrected. FT-IR spectra were taken on anBruker FT-IR Opus Spectroscopic Software Version 2.0 (Bruker Instruments Inc., USA) from 4000-400 cm^{-1} using KBr discs. ^{1}H-NMR spectra were recorded at 400 MHz in $CDCl_3$ using a Bruker/Avance 400 instrument (Bruker Instruments Inc., USA). Chemical shifts were measured in δ (ppm) unit relative to tetramethylsilane (TMS). ESI-MS spectra were recorded on a Jeol SX 102/DA-6000 Mass Spectrometer (Jeol Ltd. Akishima, Tokyo, Japan). Elemental analysis was performed on Vario EL III Elemental Analyser (Elementar, Germany) using Sulfanilamide as standard. All chemicals were purchased from Aldrich, E Merck, Spectrochem, CDH, Himedia, FinarorAvraIndia. Solvents were of reagent grade and were purified and dried by standard procedure. Reactions were monitored using Thin-layer chromatography on Silica Gel F 254 plates (Merck) with visualization by UV (254 nm) chamber.

EXPERIMENTAL

Chemistry:

Synthesis of 3,5-diarylsubstituted isoxazolines (IS 01-10): A mixture of 1-[4-(1H-pyrrol-1-yl)phenyl] ethanone chalcones (0.001moles) and hydroxyl amine (0.001moles) were dissolved in sodium acetate in glacial acetic acid (20ml) reflux it for 6hr (Voskiene and Mickevicius, 2009). After that added the solution to the cold water. The mixture was kept for 24hours and it was acidified with 1:1 HCl and water, then it was filtered through vacuum filter by washing with water. Progress of the reaction was monitored by the TLC. The precipitated solid was dried and recrystallized. The obtained solid purified by column chromatography from a mixture of ethyl acetate and hexane (1:1). The structure, physico-chemical characterization of compounds (IS 01-10) were presented in Table 1.

Table.1. Physical data of 3,5-diarylsubstituted isoxazolines (IS 01-10)

S. No	Compound	Substituents					Molecular Formula	Mol. wt	M.P. (°C)	Yield (%)
		R^2	R^3	R^4	R^5	R^6				
1	IS 01	-O-CH_3	-H	-O-CH_3	-H	-O-CH_3	$C_{22}H_{22}N_2O_4$	378.42	134-137	85
2	IS 02	-H	-O-CH_3	-O-CH_3	-O-CH_3	-H	$C_{22}H_2N_2O_4$	378.42	137-139	84
3	IS 03	-H	-H	-S-CH_3	-H	-H	$C_{20}H_{18}N_2OS$	334.43	133-136	78
4	IS 04	-H	-H	-CF_3	-H	-H	$C_{20}H_{15}F_3N_2O$	356.34	141-146	85
5	IS 05	-H	-H		-H	-H	$C_{26}H_{22}N_2O_2$	394.47	134-135	87
6	IS 06	-CF_3	-H	-H	-H	-H	$C_{20}H_{15}F_3N_2O$	356.34	155-157	94
7	IS 07	-Cl	-H	-H	-H	-F	$C_{19}H_{14}ClFN_2O$	340.78	135-156	89
8	IS 08	-H	-H		-H	-H	$C_{23}H_{25}N_3O$	359.46	145-146	84
9	IS 09	-H	-H	-C_2H_5	-H	-H	$C_{21}H_{20}N_2O$	316.40	148-149	86
10	IS 10	-OH	-H	-H	-H	-H	$C_{19}H_{16}N_2O_2$	304.12	145-147	84

5-[4-(1H-pyrrol-1-yl)phenyl]-3-(2,4,6-trimethoxyphenyl)-4,5-dihydro-1,2-oxazole (IS-01): IR (KBr, cm^{-1}): 3105.32, 3048.51, 2936.16,1660.76, 1605.24, 1583.83, 1461.77, 1371.05, 1246.25; H^1 NMR($CDCl_3$, δ, ppm):7.14 (2H, d, C-2,5 of pyrrole), 7.30 (2H, d, C-3,4 of pyrrole), 7.48-7.78 (6H, m, Ar-H), 3.85 (2H, d, C-4 of isoxazole), 5.93 (1H, t, C-5 of isoxazole), 3.83 (9H, s, 3-OCH3) MS m/z: 379 [M+1]. Analysis Calculated for $C_{22}H_{22}N_2O_4$: C, 69.83; H, 5.86; N, 7.40. Found: C, 69.78; H, 5.91; N, 7.36.

5-[4-(1H-pyrrol-1-yl)phenyl]-3-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1,2-oxazole (IS-02): IR (KBr, cm^{-1}): 3119.04, 2937.74, 2834.04, 1661.12, 1610.32, 1588.75, 1486.59, 1420.91, 1248.15, 828.23; H^1 NMR($CDCl_3$, δ, ppm):7.15 (2H, d, C-2,5 of pyrrole), 7.32 (2H, d, C-3,4 of pyrrole), 7.42-7.82 (6H, m, Ar-H), 3.85 (2H, d, C-4 of isoxazole), 5.93 (1H, t, C-5 of isoxazole), 3.83 (9H, s, 3-OCH_3)MS m/z: 379 [M+1]. Analysis Calculated for $C_{22}H_2N_2O_4$: C, 69.83; H, 5.86; N, 7.40. Found: C, 69.87; H, 5.90; N, 7.36.

5-[4-(1H-pyrrol-1-yl)phenyl]-3-(4-methylthiophenyl)-4,5-dihydro-1,2-oxazole (IS-03): IR (KBr, cm^{-1}): 3144.43, 3051.37, 2926.41,1657.87, 1600.46, 1588.82, 1491.32,1426.08, 1333.18, 1252.25; H^1 NMR ($CDCl_3$, δ, ppm):7.15 (2H, d, C-2,5 of pyrrole), 7.35 (2H, d, C-3,4 of pyrrole), 7.45-7.86 (8H, m, Ar-H), 3.85 (2H, d, C-4 of isoxazole), 5.93 (1H, t, C-5 of isoxazole), 2.53 (3H, s, -CH_3);MS m/z: 335 [M+1]. Analysis Calculated for $C_{20}H_{18}N_2OS$: C, 71.83; H, 5.42; N, 8.38; S, 9.59. Found: C, 71.88; H, 5.47; N, 8.35; S, 9.56.

5-[4-(1H-pyrrol-1-yl)phenyl]-3-(4-trifluoromethylphenyl)-4,5-dihydro-1,2-oxazole (IS-04): IR (KBr, cm^{-1}): 2971.04, 2922.04, 2866.04, 1661.12, 1610.52, 1588.75, 1455.99, 1325.03, 828.23;H^1 NMR($CDCl_3$, δ, ppm): 7.15 (2H, d, C-2,5 of pyrrole), 7.22 (2H, d, C-3,4 of pyrrole), 7.31-7.96 (8H, m, Ar-H), 3.85 (2H, d, C-4 of isoxazole), 5.93 (1H, t, C-5 of isoxazole); MS m/z: 357 [M+1]. Analysis Calculated for $C_{20}H_{15}F_3N_2O$: C, 67.41; H, 4.24; N, 7.86. Found: C, 67.38; H, 4.27; N, 7.91.

5-[4-(1H-pyrrol-1-yl)phenyl]-3-(4-benzyloxyphenyl)-4,5-dihydro-1,2-oxazole (IS-05): 3115.98, 2931.65, 1657.87, 1600.46, 1451.59, 1347.88, 1256.23, H^1 NMR ($CDCl_3$, δ, ppm):7.12 (2H, d, C-2,5 of pyrrole), 7.22 (2H, d, C-3,4 of pyrrole), 7.32-7.91 (13H, m, Ar-H), 3.85 (2H, d, C-4 of isoxazole), 5.93 (1H, t, C-5 of isoxazole), 5.16 (2H, s, -OCH_2-); MS m/z: 395 [M+1]. Analysis Calculated for $C_{26}H_{22}N_2O_2$: C, 79.16; H, 5.62; N, 7.10. Found: C, 79.11; H, 5.65; N, 7.14.

5-[4-(1H-pyrrol-1-yl)phenyl]-3-(2-trifluoromethylphenyl)-4,5-dihydro-1,2-oxazole (IS-06): IR (KBr, cm^{-1}): 2971.98, 2834.04, 1649.38, 1598.05, 1486.59, 1420.91, 1376.52, 1370.02, 1326.02; H^1 NMR($CDCl_3$, δ, ppm):7.15 (2H, s, C-2,5 of pyrrole), 7.26 (2H, d, C-3,4 of pyrrole), 7.30-7.26 (8H, m, Ar-H), 3.85 (2H, d, C-4 of isoxazole), 5.93 (1H, t, C-5 of isoxazole); MS m/z: 357 [M+1]. Analysis Calculated for $C_{20}H_{15}F_3N_2O$: C, 67.41; H, 4.24; N, 7.86. Found: C, 67.49; H, 4.20; N, 7.81.

5-[4-(1H-pyrrol-1-yl)phenyl]-3-(2-chloro, 6-fluorophenyl)-4,5-dihydro-1,2-oxazole (IS-07): IR (KBr, cm^{-1}): 3116.55, 3025.22, 2969.61, 1649.38, 1598.05, 1479.27, 1376.52, 1255.26; H^1 NMR($CDCl_3$, δ, ppm):7.10 (2H, d, C-2,5 of pyrrole), 7.21 (2H, d, C-3,4 of pyrrole), 7.24-7.90 (7H, m, Ar-H), 3.85 (2H, d, C-4 of isoxazole), 5.93

(1H, t, C-5 of isoxazole) ;MS m/z: 341 [M+1]. Analysis Calculated for $C_{19}H_{14}ClFN_2O$: C, 66.97; H, 4.14; N, 8.22. Found: C, 66.91; H, 4.21; N, 8.19.

5-[4-(1H-pyrrol-1-yl)phenyl]-3-(4-diethylaminophenyl)-4,5-dihydro-1,2-oxazole (IS-08): IR (KBr, cm^{-1}): 3364.23, 3119.04, 2937.74,1661.12, 1588.75, 1600.32, 1587.08, 1486.59, 1420.91, 828.23; H^1 NMR($CDCl_3$, δ, ppm):7.14 (2H, d, C-2,5 of pyrrole), 7.22 (2H, d, C-3,4 of pyrrole), 7.32-8.22 (8H, m, Ar-H), 3.85 (2H, d, C-4 of isoxazole), 5.93 (1H, t, C-5 of isoxazole), 2.52 (6H, t, 2-CH_3), 3.41 (4H, q, 2-CH_2-); MS m/z: 360 [M+1]. Analysis Calculated for $C_{23}H_{25}N_3O$: C, 76.85; H, 7.01; N, 11.69. Found: C, 76.81; H, 7.05; N, 11.63.

5-[4-(1H-pyrrol-1-yl)phenyl]-3-(4-ethylphenyl)-4,5-dihydro-1,2-oxazole (IS-09): IR (KBr, cm^{-1}): 3144.43, 3051.37 2926.41, 1657.87, 1600.46, 1588.82, 1491.32, 1491.32, 1333.18;H^1 NMR($CDCl_3$, δ, ppm):7.15 (2H, d, C-2,5 of pyrrole), 7.26 (2H, d, C-3,4 of pyrrole), 7.32-8.49 (8H, m, Ar-H), 3.85 (2H, d, C-4 of isoxazole), 5.93 (1H, t, C-5 of isoxazole), 1.25 (3H, t, -CH_3), 2.60 (2H, q, -CH_2-); MS m/z: 317 [M+1]. Analysis Calculated for $C_{21}H_{20}N_2O$: C, 79.72; H, 6.37; N, 8.85. Found: C, 79.65; H, 6.32; N, 8.92.

5-[4-(1H-pyrrol-1-yl)phenyl]-3-(2-hydroxyphenyl)-4,5-dihydro-1,2-oxazole (IS-10): IR (KBr, cm^{-1}): 3410.25, 3123.41, 2942.19, 1658.95, 1602.24,1587.10,1486.58, 756.23; H^1 NMR($CDCl_3$, δ, ppm):7.18 (2H, d, C-2,5 of pyrrole), 7.28 (2H, d, C-3,4 of pyrrole), 6.92-7.52 (8H, m, Ar-H), 3.85 (2H, d, C-4 of isoxazole), 5.93 (1H, t, C-5 of isoxazole), 5.35 (1H, s, -OH); MS m/z: 305 [M+1]. Analysis Calculated for $C_{19}H_{16}N_2O_2$: C, 74.98; H, 5.30; N, 9.20. Found: C, 74.93; H, 5.34; N, 9.26.

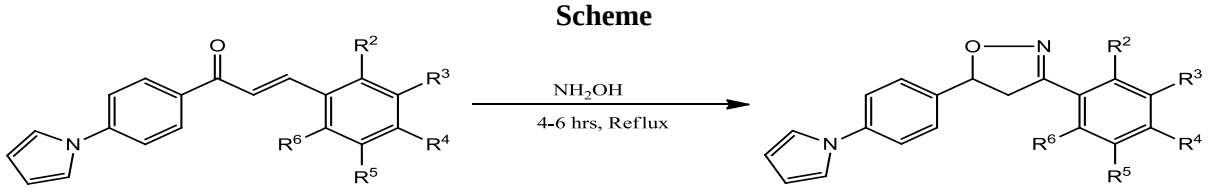
Antimicrobial activity: The MIC for antibacterial activity of synthesized compounds was determined by disc diffusion method by determining zone of inhibition (mm) for each compound using nutrient agar medium, and streptomycin was used as standard (Kumar, 2002). The stock solution of test compounds was prepared in dimethyl sulfoxide (DMSO) and sterilized by membrane filtration method using 0.22-µm pore size polycarbonate sterile membrane filters. The stock solution diluted to produce a concentration of 50µg/disc, 100µg/disc, 150µg/disc and used for the study. The total four bacterial strains used for screening antibacterial activity were *Staphylococcus aureus* (MTCC 96), *Bacillus subtilis* (MTCC 441), *E. coli* (MTCC 443), *Pseudomonas vulgaris* (MTCC 2421). A standard protocol was followed to evaluate antibacterial activity. The sterile filter papers (6 mm) having a capacity to hold 10 ul of solution were immersed in compounds under study and dried. The dried sterile filter papers (6mm) with varying concentrations of the test compounds placed over the solidified agar media, incubated at 37^0C for 24 hours. After incubation zone of inhibition measured for test and standard compounds.

Cytotoxic studies: The invitro cytotoxicity assay of target compounds was carried out using MTT assay (Rajanarendar, 2012). Stock solutions of the drugs were prepared in DMSO and diluted to produce a final concentration of < 2% DMSO (V/V), a concentration which is non-toxic to cell proliferation. The human tumour cell line panel constituted three cancer cell lines including breast (MCF-7), colon (HT-29) and prostate (DU-145). Cell lines were obtained from National Centre for Cell Science (NCCS), Pune, India. Cells were grown in RPMI-1640 containing fetal bovine serum (5%) and L-glutamine (2 mM). These cell lines were incubated with five concentrations of final compounds in a humidified atmosphere at 37^0C containing 5% CO_2. After 24 h incubation, the absorbances were read at 540 nm and used to plot dose-response curve. Three response parameters, TGI (total growth inhibition), LC_{50} and IC_{50} were calculated for each cell line.

3. RESULTS AND DISCUSSION

Chemistry: Target compounds, Compounds IS 01-10 were synthesized following the reaction sequence outlined in Scheme.A series of 3,5-diarylsubstituted isoxazolines synthesized by the condensation of 1-[4-(1*H*-pyrrol-1-yl) phenyl]ethanone chalcones with hydroxylamine. The structure of the products, IS 01-10 was established by physico-chemical and spectroscopic analysis. The IR spectra of IS 01-10 showed bands at 3150-2900 cm^{-1} (=C-H & -C-H), 1300-1500 cm^{-1} (C-C) and 1640-1550 cm^{-1} (C-N), stretching vibrations of isoxazole ring occur in the region 1300-1600cm^{-1}. The ^{1}H-NMR spectra of the synthesized compounds gave further support for the isoxazolestructure.The isoxazole C_3-H appeared at δ 8.2-8.3, the C_4-H appeared between δ6.31-6.5 while C_5-H appeared at δ 8.4-8.6. The characteristic doublets were observed at δ 7.10 ppm and 7.36 indicates the presence of pyrrole ring protons, the above statement confirms the formation of 3,5-diarylsubstituted isoxazolines. Other aromatic proton signals were appeared at δ 6.0-8.0 ppm. The parent ion peak appeared on the positive mode in the mass spectrum of all the compounds further confirms the structure of 3,5-diarylsubstituted isoxazolines.

Scheme



1 **2 (IS 01-10)**

Antimicrobial activity: All the ten derivatives (IS 01–10) were evaluated for their *in vitro* antimicrobial activity by using disc diffusion method against non-pathogenic strains of (*Bacillus subtilis & Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli & Pseudomonas vulgaris*). The results are showed in Table 2. From the results, the data reveals that amongst all the synthesized compounds (IS 01–10), compounds IS-04, 06 and IS-07 were exhibited good activity against Gram positive bacteria (*Bacillus subtilis & Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli & Pseudomonas vulgaris*) when compared to standard (streptomycin), which was statistically significant. Among all, compound IS-06showing greater inhibitory activity against all tested organisms employedwith zones inhibition of 35 to 22 mm at a concentration of 150µg/ml.

Table.2. Antimicrobial activity of 3,5-diarylsubstituted isoxazolines (IS 01-10)

Compound	Zone of Inhibition (at ug/ml; mm)											
	Bacillus subtilis			***Staphylococcus aureus***			***Escherichia coli***			***Pseudomonas vulgaris***		
	50	**100**	**150**	**50**	**100**	**150**	**50**	**100**	**150**	**50**	**100**	**150**
IS 01	14	17	21	15	16	22	11	15	22	12	16	21
IS 02	17	19	23	17	19	24	15	16	24	14	18	23
IS 03	8	12	15	9	13	13	9	14	16	8	13	16
IS 04	22	24	27	22	25	28	21	24	32	21	25	30
IS 05	10	14	17	10	14	16	10	15	18	9	14	19
IS 06	23	25	31	25	28	32	22	26	35	25	26	32
IS 07	21	23	26	22	26	27	20	21	29	20	22	29
IS 08	5	7	8	5	8	8	6	8	9	6	8	10
IS 09	18	20	23	19	21	25	16	14	25	15	21	24
IS 10	6	8	12	6	9	10	7	12	14	6	10	13
STREPTOMYCIN	26	28	32	28	30	36	28	32	38	28	34	36

Cytotoxic studies: Final compounds (IS 01–10) were screened for their *invitro* cytotoxicity against three human cancer cell lines including breast (MCF-7), colon (HT-29) and prostate (DU-145). MTT assay utilized for the screening. All the compounds synthesized displayed significant cytotoxic activity in micromolar range, compounds IS-02found to the potent one with IC_{50} values of 18 µg/mL, 23 µg/mL and 20 µg/mL against MCF-7, HT-29 & DU-105 cell lines respectively. Compound IS-01 was next in order with IC_{50} values of 24 µg/mL, 38 µg/mL and 28 µg/mL against MCF-7, HT-29 & DU-105 cell lines respectively. The results are showed in Table.3.

Table.3. Cytotoxic activity of 3,5-diarylsubstituted isoxazolines (IS 01-10)

S. No	Compound	Cell line		
		Breast cancer (MCF-7)	***Colon cancer (HT-29)***	***Prostate cancer (DU-145)***
1	IS 01	24±1	38±2	28±2
2	IS 02	18±2	23±1	20±1
3	IS 03	76±1	84±2	54±2
4	IS 04	178±1	156±1	117±1
5	IS 05	32±1	40±1	36±2
6	IS 06	195±2	174±1	154±1
7	IS 07	150±1	138±2	97±1
8	IS 08	44±1	62±1	46±2
9	IS 09	122±2	124±2	73±1
10	IS 10	108±1	106±1	65±2
16	ADRIAMYCIN	12 ± 1	9 ± 1	5 ± 1

4. CONCLUSION

The 3,5-diarylsubstituted isoxazolines (IS 01-10) were synthesized & characterized by spectral methods (IR, NMR & MS)and evaluated for antimicrobial and cytotoxicactivity. From the results, the compound IS-02, 04 & IS-06 exhibited significant biological activity with reference to standard drugs.

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REFERENCES

Ahmad G, Mishra PK, Gupta P, Yadav PP, Tiwari P, Tamrakar AK, Srivastava AK, Maurya R, Synthesis of novel benzofuran isoxazolines as protein tyrosine phosphatase 1B inhibitors, Bioorganic & Medicinal Chemistry Letters, 16 (8), 2006, 2139-2143.

Ankhiwala MD, Hathi MV, Synthesis and Antibacterial Activity of Some 1-Phenyl-3, 5-diaryl-2-pyrazolines and 3, 5-Diaryl-2-isoxazolines, Chem Inform, 26 (38), 1995.

Dannhardt G, Kiefer W, Krämer G, Maehrlein S, Nowe U, Fiebich B, The pyrrole moiety as a template for COX-1/COX-2 inhibitors, European Journal of Medicinal Chemistry, 35 (5), 2000, 499-510.

Groutas WC, Chong LS, Epp JB, Venkataraman R, Brubaker MJ, Stanga MA, Kim EH, Keller CE, Inhibition of human leukocyte elastase and cathepsin G by isoxazoline derivatives, Bioorganic & Medicinal Chemistry Letters, 2 (12), 1992, 1571-1574.

Habeeb AG, Praveen Rao PN, Knaus EE, Design and synthesis of 4, 5-diphenyl-4-isoxazolines: novel inhibitors of cyclooxygenase-2 with analgesic and anti-inflammatory activity, Journal of Medicinal Chemistry, 44 (18), 2001, 2921-2927.

Huisgen R, 1, 3-dipolar cyclo additions. Past and future, Angewandte Chemie International Edition in English, 2 (10), 1963, 565-598.

Kachhadia VV, Patel MR, Joshi HS, Synthesis of isoxazoles and cyanopyridines bearing benzo (b) thiophene nucleus as potential antitubercular and antimicrobial agents, Journal of Sciences Islamic Republic of Iran, 15 (1), 2004, 47-51.

Kai H, Matsumoto H, Hattori N, Takase A, Fujiwara T, Sugimoto H, Anti-influenza virus activities of 2-alkoxyimino-n-(2-isoxazolin-3-ylmethyl) acetamides, Bioorganic & Medicinal Chemistry Letters, 11 (15), 2001, 1997-2000.

Kedar RM, Vidhale NN, Chincholkar MM, Synthesis of new heterocycles and their antimicrobial study, Oriental Journal of Chemistry, 13, 1997, 143-148.

Kumar RU, Begum VH, Antimicrobial studies of some selected medicinal plants, Ancient Science of Life, 21 (4), 2002, 230.

Rajanarendar E, Raju S, Reddy MN, Krishna SR, Kiran LH, Reddy AR, Reddy YN, Multi-component synthesis and *in vitro* and *in vivo* anticancer activity of novel arylmethylene bis-isoxazolo [4, 5-b] pyridine-N-oxides, European Journal of Medicinal Chemistry, 50, 2012, 274-279.

Sharma V, Sharma KV, Synthesis and biological activity of some 3, 5-diarylisoxazoline derivatives: reaction of substituted chalcones with hydroxylamine hydrochloride, E-Journal of Chemistry, 7 (1), 2010, 203-209.

Sudhir P, Rajashree C, Ashok B, Synthesis and biological evaluation of mannich bases of isoxazoline derivatives as novel anti-microbial agents, E-Journal of Chemistry, 9 (4), 2012, 1760-1772.

Tangallapally RP, Sun D, Budha N, Lee RE, Lenaerts AJ, Meibohm B, Lee RE, Discovery of novel isoxazolines as anti-tuberculosis agents, Bioorganic & Medicinal Chemistry Letters, 17 (23), 2007, 6638-6642.

Velikorodov AV, Sukhenko LT, Synthesis and antimicrobial activity of 3, 5-disubstituted isoxazolines and isoxazoles with carbamate groups, Pharmaceutical Chemistry Journal, 37 (1), 2003, 22-24.

Voskiene A, Mickevicius V, Cyclization of chalcones to isoxazole and pyrazole derivatives, Chemistry of heterocyclic compounds, 45 (12), 2009.