Organic Chemistry
ISSN: 0974-7516

Organic Chemistry: An Indian Journal

Research | Vol12 Iss 2

Synthesis and Pharmacological Evaluation of Some 3-(2-Fluorneyl)-5-(Substituted Phenyl)-4,5-Dihydroisoxazoles

Ganesamoorthy Thirunarayanan*

Department of Chemistry, Annamalai University, Annamalainagar-608002, India

***Corresponding author:** Peter Ghosh, Annamalai University, Annamalainagar-608002, India, Tel: 04144 238 282 E-mail: drgtnarayanan@gmail.com

Received: 06 June, 2016; **Accepted:** 20 June, 2016; **Published:** 25 June, 2016

Abstract

Totally twenty seven 3,5-substituted 4,5-dihydro isoxazole derivatives including 3-(2-fuorneyl)-5-(substituted phenyl)-4,5-dihydroisoxazole have been synthesized by fly-ash:H2SO4 catalyzed intramolecular cycloaddition of hydroxylamine hydrochloride and aryl chalcones under solvent-free conditions. The yields of the isoxazoles are more than 90%. The synthesized isoxazole were characterized by their physical constants and spectral data. The anti-microbial and antioxidant activities of the synthesized isoxazoles have been evaluated using a variety of bacterial and fungal species and DPPH radical scavenging methods.

Keywords: ***3, 5-Disubstituted aryl 4,5-dihydro isoxazoles; Solvent-free synthesis; Fly-ash: H2SO4; Antimicrobial activities; Antioxidant activity.***

.

Introduction

Isoxazole and it derivatives are an important five membered heterocyclic family. They are in the use of natural product synthesis [1] and occurrence from pharmaceuticals substrate [2]. Numerous solvent assisted and solvent-free synthetic methods reported in the literature for the synthesis of isoxazole derivatives. The solvent assisted and solvent-free [3+2] cycloaddition of alkynes and nitriles oxides and its alcohols [3-7], C-H activation/[4+1] annulation [8] and side chain rearrangements [9] were employed for synthesis of isoxazoles. Chalcones also utilized for synthesis of isoxazole derivatives by cyclization with hydroxylamine hydrochloride in presence of catalysts under solvent-assisted or solvent-free methods [10], have synthesized some novel aryl isoxazoles by cyclization of substituted phenyl chalcones with hydroxylamine hydrochloride. Indole based isoxazole derivatives were synthesized by cyclization of indolyl chalcones and hydroxylamine hydrochloride [11]. A series of 3-(2-furyl)-5-(substituted phenyl)-4,5-dihydro isoxazoles [12] were synthesized by cyclization of 2-furyl chalcones and hydroxylamine hydrochloride. Some benzothiophene based isoxazole derivative were synthesized using cyclization of benzothiophene chalcones and hydroxylamine hydrochloride by Kachhadia et al. [13]. Many kinds of isoxazole derivatives possess various biological activities due to hetero atom O, N, double bond and polar groups presents in the ring [13]. The important biological activities of isoxazoles are antibacterial [13-18] antifungal [1,15-18], antitubercular [13,18], antioxidant [17,19,20], anti-inflammatory [20,21], ulcerogenicity [21], antihypertensive [22], growth promoting effect of plants [23], immunological activity [24], nicotinic receptor binding [25], molecular docking [26], DNA binding

[16], photonuclease activity [16], *invitro* calcium channel antagonist activity [17], antianalgesic [17], anti-platelet [17] anti HIV [17] and anthelmintic [26] activities. These 3,5-disubstituted 4,5-dihydro isoxazole derivatives were used as starting materials for synthesis of copper (II) complexs [27]. The experimental and theoretical study on the photo physical, physiochemical [28] and electrochemical behavior of isoxazole [27] were reported. Within the above view, the synthesis of 3-(9*H*-2-fluorenyl)-5-(substituted phenyl)-4,5-dihydro isoxazole derivatives has not been reported. Hence, the author have synthesized 3-(9*H*-2-fluorenyl)-5-(substituted phenyl)-4,5-dihydro isoxazoles and evaluated their pharmacological activities, including antimicrobial and antioxidant activities using the appropriate microbial strains with Bauer-Kirby [29] and DPPH radical scavenging [30] methods.

Experimental

General

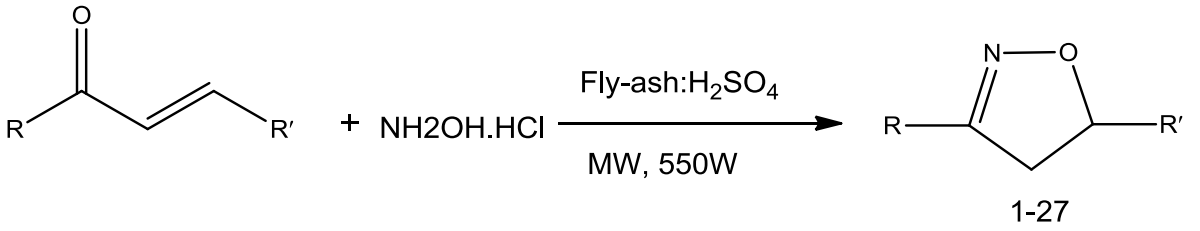
All chemicals were procured from Sigma-Aldrich and E. Merck. Melting points of isoxazoles were determined in open glass capillaries on a Mettler FP51 melting point apparatus and are uncorrected. Infrared spectra (KBr, 4000-400 cm^{-1}) were recorded on Thermo scientific Nicolet iS5, US-made Fourier transform spectrophotometer. The NMR spectra of selective compounds were recorded on a Bruker AV 300 spectrometer operating at 500 MHz for ^{1}H NMR spectra and 125 MHz for ^{13}C NMR spectra in $CDCl_3$ solvent using TMS as internal standard. Electron impact and chemical ionization mode FAB^+ mass spectra were recorded with a Shimadzu spectrometer.

Synthesis of 9H-2-fluorenyl chalcones

The 9*H*-2-fluorenyl chalcones were synthesized as described in reference [31].

General procedure for synthesis of 3-(9H-2-fluorenyl)-5-(substituted phenyl)-4,5-dihydro isoxazoles

Appropriate equimolar quantities of 9*H*-2-fluorenyl chalcones (2 mmol), hydroxylamine hydrochloride (2 mmol) and 0.4 g of fly-ash:H_2SO_4 catalyst were taken in a 50 ml borosil beaker thoroughly mixed and closed with watch glass. The reaction mixture was subjected to microwave heating in a microwave oven at 450 W (Samsung Grill, GW73BD model, 230 V, 50 Hz, 100-750 W) for 4-7 min (Scheme 1). Progress of the reaction was monitored by thin-layer chromatography. Dichloromethane (10 mL) was added and the extract was separated by filtration. The filtrate was washed with water, brine (10 mL), dried over anhydrous Na_2SO_4 and concentrated to give a solid product. The crude product was further purified by recrystallization with ethanol.



1-27

Entry 19: R= 9H-fluorenyl
R′=Phenyl

Scheme 1: Synthesis of 3,5-diaryl-4,5-dihydro isoxazole derivatives by fly-ash:H_2SO_4 assisted solvent-free cyclization of 1,3-diaryl chalcones and hydroxylamine hydrochloride.

Measurement of anti-microbial activities

The antimicrobial activities of prepared isoxazoles (compounds 19-27) have been evaluated by measuring the mm of zone of inhibition of the compounds against the bacterial and fungal strains. In this present study the author have chosen two gram positive pathogenic strains *Staphylococcus aureus*, *Entrocccus faecalis* while *Escherichia coli*, *Klebsiella species*, *Psuedomonas* and *Proteus vulgaris* were the gram negative strains. The disc diffusion technique will be followed using the Kirby-Bauer[29] method, at a concentration of 250 µg/mL with Ampicillin and Streptomycin taken as the standard drugs. For the study of antifungal activities of all isoxazoles using *Candida albicans* as the fungal strain and the disc diffusion technique was followed for the antifungal activity while the two other stains *Penicillium* species and *Aspergillus niger*, the dilution method [29] will be used. The drugs dilution will be 50 µg/mL. Grisseofulvin is taken as the standard drug.

Measurement of antibacterial activity

Antibacterial sensitivity assay was performed using Kirby-Bauer [29] disc diffusion technique. In each Petri plate about 0.5 ml of the test bacterial sample was spread uniformly over the solidified Mueller Hinton agar using sterile glass spreader. Then the discs with 5 mm diameter made up of Whatmann No.1 filter paper, impregnated with the solution of the compound were placed on the medium using sterile forceps. The plates were incubated for 24 hours at 37^{o}C by keeping the plates upside down to prevent the collection of water droplets over the medium. After 24 hours, the plates were visually examined and the diameter values of the zone of inhibition were measured. Triplicate results were recorded by repeating the same procedure.

Measurement of Antifungal sensitivity assay

Antifungal sensitivity assay was performed using Kirby-Bauer [29] disc diffusion technique. PDA medium was prepared and sterilized as above. It was poured (ear bearing heating condition) in the Petri-plate which was already filled with 1 mL of the fungal species. The plate was rotated clockwise and counter clock-wise for uniform spreading of the species. The discs were impregnated with the test solution. The test solution was prepared by dissolving 15 mg of the isoxazole in 1 mL of DMSO solvent. The medium was allowed to solidify and kept for 24 h. Then the plates were visually examined and the diameter values of zone of inhibition were measured. Triplicate results were recorded by repeating the same procedure.

Antioxidant activity

The antioxidant activities of all synthesized isoxazoles (compounds 19-27) have been evaluated by the DPPH radical scavenging effect [30-34]. The 0.1 M acetate will be prepared by dissolving 1.64 g of sodium acetate in 15 mL of water and 150 µL of acetic acid. The final volume will be adjusted to 20 mL by adding water. The 0.2 mmol of DPPH solution will be prepared by dissolving 3.9 g of DPPH in 50 mL of ethanol. α- Tocofereol (1 mg in 10 mL of ethanol) solution was prepared. A series of test tubes will be arranged with 1.0 mL of buffer solution mixed with 0.5 mL of DPPH solution. A series of various concentrations of synthesized isoxazoles and α-Tocofereol (1 µg in 1 ml of ethanol) will be added to each tube and mixed well. After 30 minutes in room temperature the absorbance of each solution will be measured by UV spectrophotometer at 517 nm. A mixture of buffer solution and ethanol used as the reference for the spectrophotometer. A graph was plotted with the weight of the compound Vs absorptions and IC50 values will be determined. The antioxidant activity will be expressed in terms of IC50 (µg/mL, concentration required to inhibit DPPH radical formation by 50%). α-Tocofereol will be used as a positive control. The radical scavenging activity was calculated as,

$$\text{DPPH radical scavenging activity} = \frac{\text{Control absorbance - Sample absorbance}}{\text{Control absorbance}} \times 100$$
$$(\%\ \text{of inhibition})$$

Results And Discussion

The synthesis of 3,5-diaryl-4,5-dihydro isoxazole derivatives by solid fly-ash: H_2SO_4 catalyzed cyclization of aryl chalcones and hydroxylamine hydrochloride were undertaken. The author obtained 3,5-diaryl-4,5-dihydro isoxazoles by cyclization *E*-enones and hydroxylamine hydrochloride using microwave irradiation under solvent-free conditions. The solid acid fly-ash: H_2SO_4 assists the intramolecular cycloaddition of nitrile oxide which is generated from the chalcones oxime followed by dehydration and proton transfer gave the isoxazoles. The chalcones oxime was formed by nucleophilic addition to carbonyl group carbon of chalcones and hydroxylamine hydrochloride. In this reaction the yield obtained was greater than 90%. The determined physical constants and mass fragments are presented in Table 1. The reusability of the catalyst in this cyclization reaction was studied with 2 mmol of 9*H*-2-fluorenyl chalcone and 2 mmol of hydroxylamine hydrochloride and is presented in Table 2 (compound **19**). The first run gave 95% yield; the second and third runs gave 94% yields, and the fourth and fifth runs gave 93%. The chalcone-containing electron-donating substituents (OCH_3) gave higher yield than electron-withdrawing (halogen and nitro) substituents. The effect of the catalyst on this reaction was studied by varying the catalyst quantity from 0.1 to 1 g. As the catalyst quantity increased from 0.1 to 1 g the percentage of product increased from 90-95%. Further increases in catalyst amount beyond 0.4 g did not increase the percentage of product. The effect of catalyst content is shown in Figure 1. The optimum quantity of catalyst was found to be 0.4 g for 0.4 g of 9*H*-2-fluorrenyl chalcones substrate. The ratio of 9*H*-2-fluorenyl chalcones hydroxylamine hydrochlorides and the fly-ash:H_2SO_4 catalyst ratio is 1:0.5:1. The effect of solvents on this reaction by conventional heating method was studied with the same quantity of reactants with methanol, dichloromethane, dioxane and tetrahydrofuran and is presented in Table 3. The highest yield was obtained in solvent-free MW irradiation method.

Table 1: The analytical physical constants and mass fragments of 3, 5-diaryl-4,5-dihydro isoxazole derivatives

Entry	R	R′	Yield (%)	Time (m)	M.W.	m.p. (°C)	Micro analysis		
							C (Cacld.)	H (Cacld.)	N (Cacld.)
1	Phenyl	Phenyl	95	4	223	144-145 (142-145) [32]	---	---	---
2	Phenyl	4-Chlorophenyl	90	4	258	188-189 (185-188) [32]	---	---	---
3	Phenyl	3-Indole	90	5	264	147-148 (147) [11]	---	---	---
4	4-Aminophenyl	2-Furyl	91	4	330	125-127 (120-125)	---	---	---

						[12]			
5	4-Aminophenyl	3-Indole	91	6	272	151-152 (152) [11]	---	---	---
6	4-Bromophenyl	2-Furyl	92	5	294	144-145 (141-145) [12]	---	---	---
7	4-Bromophenyl	3-Indole	91	5	343	119-120 (119) [11]	---	---	---
8	4-Chlorophenyl	4-Chlorophenyl	92	4	294	138-139 (135-138) [32]	---	---	---
9	4-Chlorophenyl	2-Furyl	92	5	250	150-152 (148-150) [12]	---	---	---
10	4-Chlorophenyl	3-Indole	92	4	299	128-129 (128) [11]	---	---	---
11	4-Fluorophenyl	2-Furyl	91	6	233	128-129 (125-128) [12]	---	---	---
12	4-Fluorophenyl	3-Indole	91	7	282	157-158 (157) [11]	---	---	---
13	4-Hydroxyphenyl	3-Indole	93	7	280	161-162 (161) [12]	---	---	---
14	4-Methoxyphenyl	3-Indole	96	6	294	183-184 (183) [11]	---	---	---
15	4-Methylphenyl	2-Furyl	95	5	229	138-139 (135-138) [12]	---	---	---

16	4-Methylphenyl	3-Indole	94	5	278	138-139 (138) [11]	---	---	---
17	4-Nitrophenyl	2-Furyl	90	4	260	120-121 (115-120) [12]	---	---	---
18	4-Nitrophenyl	3-Indole	90	5	309	167-168 (187) [11]	---	---	---
19	9*H*-2-Fluorenyl	Phenyl	95	6	311	101-102	84.80 (84.86)	5.42 (5.50)	4.43 (4.50)
20	9*H*-2-Fluorenyl	3-Aminophenyl	93	5	326	97-98	80.98 (80.96)	5.48 (5.56)	8.49 (8.58)
21	9*H*-2-Fluorenyl	3-Chlorophenyl	91	5	345	114-116	76.43 (76.41)	4.59 (4.66)	4.01 (4.05)
22	9*H*-2-Fluorenyl	4-Chlorophenyl	91	5	345	123-124	76.46 (76.41)	4.54 (4.66)	4.06 (4.05)
23	9*H*-2-Fluorenyl	4-Dimehylaminophenyl	92	7	354	89-90	81.36 (81.33)	6.18 (6.26	7.86 (7.90)
24	9*H*-2-Fluorenyl	4-Hydroxyphenyl	93	6	327	94-96	80.75 (80.71)	5.15 (5.23)	4.20 (4.28)
25	9*H*-2-Fluorenyl	4-Methoxyphenyl	96	6	341	117-118	80.95 (80.92)	5.04 (5.11)	4.06 (4.10)
26	9*H*-2-Fluorenyl	3-Nitrophenyl	90	4	356	132-133	74.20 (74.15)	4.48 (4.53)	7.78 (7.86)
27	9*H*-2-Fluorenyl	4-Nitrophenyl	90	4	356	121-122	74.19 (74.15)	4.52 (4.53)	7.84 (7.86)

Table 2: The reusability of the catalyst on the yield of cyclization of aryl chalcones and hydroxylamine hydrochloride (entry19).

Run	**1**	**2**	**3**	**4**	**5**
Yield	95	94	94	93	93

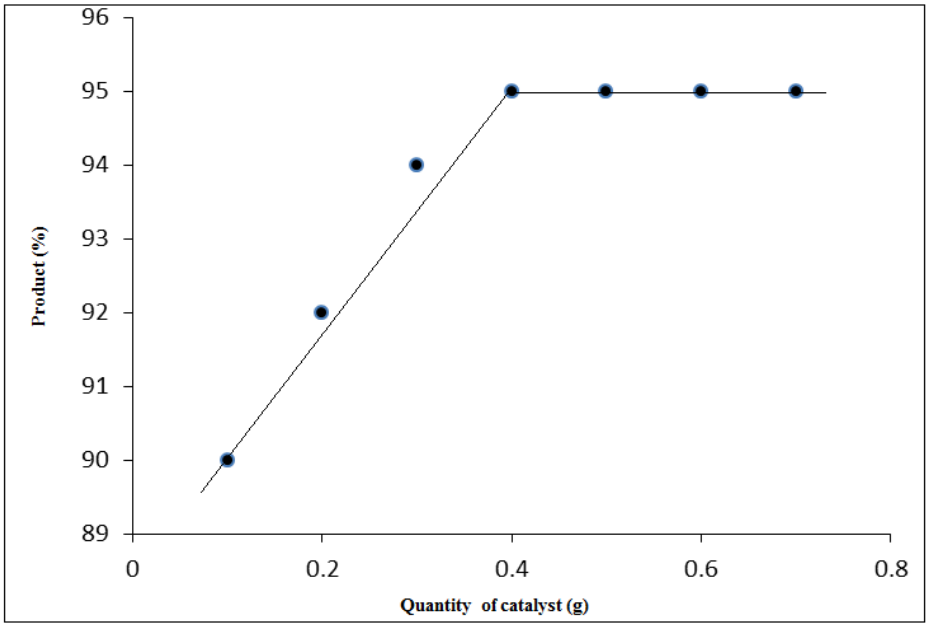


Figure 1: The effect of catalyst loading.

Table 3: The effect of solvents on yield of cyclization of aryl chalcones and hydroxylamine hydrochloride (entry19).

Solvent	**Ethanol**	**Methanol**	**Dichloromethane**	**Dioxane**	**Tetrahydrofuran**
Yield (%)	71	68	72	70	72

The infrared, NMR and mass spectral data of selective compounds (**19-27**) are as follows.

3-(9*H*-2-Fluorenyl)-5-phenyl-4,5-dihydroisoxazole (**19**): IR (KBr, cm^{-1}): ν =3017, 2983, 1582, 1448, 1416, 1082, 738; ^{1}H NMR (500 MHz, $CDCl_3$, 25°C, TMS): δ=3.086(dd, 1H, H_4, J=7 and 9.5 Hz), 3.791(dd, 1H, H_4', J=12 and 4.5 Hz), 5.251(dd, 1H, H_5, J=7 and 5 Hz), 4.322(s, 2H, CH_2, fluorene ring), 6.623-7.381 (m, 12H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$, 25°C, TMS): δ=155.60(C_3), 43.66(C_4), 84.68(C_5), 34.61(CH_2, fluorene ring), 128.75-146.52 (Ar-C); Mass: (m/z)=311[M^+], 281, 259, 234, 197, 165, 146, 114, 77, 69, 52, 51, 30, 14.

3-(9*H*-2-Fluorenyl)-5-(3-aminophenyl)-4,5-dihydroisoxazole (**20**): IR (KBr, cm^{-1}): ν=3456, 3005, 2980, 1604, 1438, 1405, 1102, 722; ^{1}H NMR (500 MHz, $CDCl_3$, 25°C, TMS): δ=3.071(dd, 1H, H_4, J=7 and 10 Hz), 3.724 (dd, 1H, H_4', J=7.5 and 4.5 Hz), 5.248(dd, 1H, H_5, J=7.5 and 10 Hz), 5.811(s, 2H, NH_2), 4.283(s, 2H, CH_2, fluorene ring), 6.673-7.321 (m, 11H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$, 25°C, TMS): δ=156.10(C_3), 43.72(C_4), 84.47(C_5), 31.13(CH_2, fluorene ring), 127.18-146.48(Ar-C); Mass: (m/z)=326[M^+], 310, 296, 274, 234, 212, 207, 165, 161, 119, 92, 92, 76, 69, 62,52, 51,16.

3-(9*H*-2-Fluorenyl)-5-(3-chlorophenyl)-4,5-dihydroisoxazole (**21**): IR (KBr, cm^{-1}): ν =2995, 2982, 1598, 1504, 1432, 1078, 825; ^{1}H NMR (500 MHz, $CDCl_3$, 25°C, TMS): δ=3.157(dd, 1H, H_4, J=6.5 and 8.5 Hz), 3.564(dd, 1H, H_4', J=5.5 and 10 Hz), 5.584(dd, 1H, H_5, J=9 and 7.5 Hz), 4.342(s, 2H, CH_2, fluorene ring), 6.611-7.351(m, 11H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$, 25°C, TMS): δ=156.33(C_3), 42.3 (C_4), 84.27(C_5), 35.16(CH_2, fluorene ring), 124.25-142.23(Ar-C); Mass: 345[M^+], 347[M^{2+}], 310, 231, 180, 151, 111, 77, 62, 52, 51, 36.

3-(9*H*-2-Fluorenyl)-5-(4-chlorophenyl)-4,5-dihydroisoxazole (**22**): IR (KBr, cm^{-1}): ν=3010, 2992, 1608, 1514, 1451, 1042, 657; ^{1}H NMR (500 MHz, $CDCl_3$, 25°C, TMS): δ=3.012(dd, 1H, H_4, J=6.5 and 10 Hz), 3.931(dd, 1H, H_4', J=12.5 and 4.5 Hz), 5.663(dd, 1H, H_5, J=7 and 5.5 Hz), 4.291(s, 2H, CH_2, fluorene ring), 6.813- 7.431(m, 11H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$, 25°C, TMS): δ=157.50(C_3), 42.08(C_4), 81.50(C_5), 32.04(CH_2, fluorene ring), 125.91-143.99(Ar-C); Mass: 345[M^+], 347[M^{2+}], 315, 310, 293, 234, 231, 180, 165, 151, 114, 111, 77, 62, 52, 51, 40, 36, 30.

3-(9*H*-2-Fluorenyl)-5-(3-dimethylaminophenyl)-4,5-dihydroisoxazole (**23**): IR (KBr, cm^{-1}): ν=3110, 2997, 1599, 1425, 1235, 1057, 785, 624; ^{1}H NMR (500 MHz, $CDCl_3$, 25°C, TMS): δ=3.101(dd, 1H, H_4, J=7 and 4.5 Hz), 3.732(dd, 1H, H_4', J=12.5 and 4.5 Hz), 5.285(dd, 1H, H_5, J=7 and 5 Hz), 4.471(s, 2H, CH_2, fluorene ring), 3.372(s, 6H, $N(CH_3)_2$), 6.713-7.384(m, 11H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$, 25°C, TMS): δ=155.82(C_3), 43.70(C_4), 84.75 (C_5), 39.74($N(CH_3)_2$), (34.75(CH_2, fluorene ring), 128.52-144.40 (Ar-C); Mass: 354[M^+], 339, 324, 310, 234, 205, 120, 189, 165, 149, 114, 105, 44, 30, 15.

3-(9*H*-2-Fluorenyl)-5-(3-hydroxyphenyl)-4,5-dihydroisoxazole (**24**): IR (KBr, cm^{-1}): ν=3510, 3002, 2984, 1588, 1512, 1465, 1124, 1024, 885, 637; ^{1}H NMR (500 MHz, $CDCl_3$, 25°C, TMS): δ=3.173(dd, 1H, H_4, J=7.5 and 9.5 Hz), 3.886(dd, 1H, H_4', J=12.5 and 4.5 Hz), 5.297(dd, 1H, H_5, J=7 and 5.5 Hz), 4.276(s, 2H, CH_2, fluorene ring), 5.132(s, 1H, OH), 7.217- 7.583(m, 11H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$, 25°C, TMS): δ=155.56(C_3), 43.53(C_4), 84.10(C_5), (31.12(CH_2, fluorene ring), 112.13-144.32(Ar-C); Mass: 327[M^+], 310, 297, 275, 234, 165, 162, 133,113, 93, 77, 52, 40, 30, 17.

3-(9*H*-2-Fluorenyl)-5-(4-methoxyphenyl)-4,5-dihydroisoxazole (**25**): IR ν=3012, 2995, 1593, 1502, 1435, 1258, 1354, 858, 647; ^{1}H NMR (500 MHz, $CDCl_3$, 25°C, TMS): δ=3.080(dd, 1H, H_4, J=4.5 and 5.5 Hz), 3.665(dd, 1H, H_4', J=7.5 and 4.5 Hz), 5.245(dd, 1H, H_5, J=7.5 and 5 Hz), 4.387, 3.806(s, 3H, OCH_3), (s, 2H, CH_2, fluorene ring), 5.132(s, 1H, OH), 7.217-7.583(m, 11H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$, 25°C, TMS): δ=155.81(C_3), 43.75(C_4), 85.28(C_5), 55.28(OCH_3), 31.57(CH_2, fluorene ring), 113.57-144.43(Ar-C); Mass: 341[M^+], 326, 310, 289, 259, 234, 165, 107, 113, 91, 69, 52, 31, 16, 15.

3-(9*H*-2-Fluorenyl)-5-(3-nitrorophenyl)-4,5-dihydroisoxazole (**26**): IR (KBr, cm^{-1}): ν=3002, 2989, 1610, 1525, 1425, 1221, 1244, 801, 710, 614; ^{1}H NMR (500 MHz, $CDCl_3$, 25°C, TMS): δ=3.154(dd, 1H, H_4, J=8 and 6.5 Hz), 3.712(dd, 1H, H_4', J=9.5 and 4.5 Hz), 5.547(dd, 1H, H_5, J=8.5and 6.5 Hz), 4.521(s, 2H, CH_2, fluorene ring), 6.841- 7.742(m, 11H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$, 25°C, TMS): δ=157.25(C_3), 44.21(C_4), 84.25(C_5), 34.35(CH_2, fluorene ring), 126.25-145.94(Ar-C); Mass: 356[M^+], 310, 242, 234, 194, 165, 122, 101, 90, 77, 52, 46, 40, 30.

3-(9*H*-2-Fluorenyl)-5-(4-nitrorophenyl)-4,5-dihydroisoxazole (**27**): IR (KBr, cm^{-1}): ν=3010, 2994, 1598, 1505, 1435, 1254, 1201, 892, 732, 612; ^{1}H NMR (500 MHz, $CDCl_3$, 25°C, TMS): δ=3.061(dd, 1H, H_4, J=7 and 9.5 Hz), 3.785(dd, 1H, H_4', J=10.5 and 4.5 Hz), 5.218(dd, 1H, H_5, J=7.5and 5 Hz), 4.681(s, 2H, CH_2, fluorene ring), 6.823- 7.781(m, 11H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$, 25°C, TMS): δ=156.93(C_3), 43.57(C_4), 83.95(C_5), 31.45(CH_2, fluorene ring), 119.34-146.93(Ar-C); Mass: 356[M^+], 326, 310, 242, 234, 205, 194, 191, 165, 151, 122, 114, 101, 90, 77, 52, 46, 30.

Antibacterial sensitivity assay

The disc-diffusion technique was followed using the Bauer-Kirby [29] method, at a concentration of 250 μg/mL [33], with ampicillin and streptomycin used as the standard drugs. The measured antibacterial activities of all isoxazoles are presented in Table 4. Compounds 21, 22 and 25 showed the maximum zone of inhibition against *Escherichia coli*, at 20-24 mm, compared to other isoxazoles such as 20, 23 and 24. These latter compounds are moderately active, with 13-19 mm zones of inhibition.

Isoxazoles 19 and 27 were active with an 8-12 mm of zone of inhibition. The parent compound 26 was inactive. The isoxazoles 20-22 and 25 were found to be effective against *S. aureus* strain with 20-24 mm of zones of inhibition. Compounds 19 and 14 are active with 13-19 mm of zones of inhibition. The isoxazole 23 was moderately active with an 8-12 mm zone of inhibition. Compounds 26 and 27 are inactive against *S. aureus*. The isoxazole derivatives 21 and 22 were shown to be more active against Pseudomonas, with greater than 20 mm zone of inhibition, while the other derivatives showed zones of inhibition between 13-19 mm. Isoxazoles 24 and 19 are moderately active with 8-12 mm of zone of inhibition. Compounds 23 and 26 were inactive against the *Pseudomonas aeruginosa* strain. Isoxazole derivatives 20, 21 and 22 were more effective against the *Klebsiella pneumoniae* strain with 20-24 mm zones of inhibition, while the parent compound 19 and 25 showed moderate activity in 13-19 mm zone of inhibition. The compounds 23 and 24 were active with an 8-12 mm zone of inhibition. Isoxazoles 26 and 27 were inactive against the *K. pneumoniae* species. The isoxazoles 21**,** 22 and 25 were active when they were screened with *Phaseolus vulgaris* with 20–24 mm zones of inhibition and compounds 23 and 24 were active with 13–19 mm zones of inhibition. The azole compounds 20 and 27 were moderately active with 8-12 mm of zone of inhibition against the *P. vulgaris* strain. Compounds 19 and 26 were inactive. The isoxazole derivatives 21 and 22 showed greater activity against *Enterococcus faecalis*, with 20-24 mm zones of inhibition. Compounds 20 and 25 were moderately active with 13-19 mm zones of inhibition. The isoxazoles 23, 24 and 19 were active with 8-12 mm zones of inhibition. Compounds 26 and 27 are inactive when it was screened against *E. faecalis.*

Table 4: Antibacterial[a], antifungal[b] and antioxidant[c] activities of 3-(9*H*-2-fluorenyl)-5-(substituted phenyl)-4,5-dihydro isoxazoles

Entry	**Antibacterial activity**						**Antifungal activity**			**Antioxidant activity (DPPH radical scavenging)**
							Disc diffusion technique (250 μg/mL)	**Drug dilution method(250 μg/mL)**		
	E. coli	***S. aures***	***P. aeruginosa***	***K. pneumoniae***	***P. vulgaris***	***E. faecalis***	***C. albicans***	***Penicillium sp.***	***A. niger***	
19	+	+	+	+	±	±	±	---	±	24.73 ± 1.18
20	+	++	+	++	±	+	+	++	++	23.11 ± 1.94
21	++	++	++	++	++	++	±	±	+	19.28 ± 1.09
22	++	++	++	++	++	++	±	±	+	22.55 ± 1.54
23	+	±	---	±	+	±	++	+	++	24.01 ± 1.65
24	+	+	±	±	+	±	±	---	±	37.87 ± 1.45
25	++	++	+	+	++	+	+	±	++	34.88 ± 1.32
26	---	---	±	±	---	±	++	++	++	12.14 ± 1.32
27	±	---	---	±	±	±	++	++	++	11.04 ± 1.82

[a] Disc size: 6.35 mm; duration: 24-45 h; standard: ampicillin (30–33 mm) and streptomycin (20–25 mm); control: methanol; –: no activity; ±: active (8–12 mm); +: moderately active (13–19 mm); ++: active (20–24 mm). [b] Standard: griseofulvin and gentamycin; duration: 72 h; control: methanol; medium: Potato dextrose agar; ++: no fungal colony; +: one fungal colony; ±: two-three fungal colonies; –: Multiple fungal colonies. [c] Standard: α-Tocopherol (39.14 ± 1.57).

Antifungal sensitivity assay

The observed antifungal activities of all prepared 3-(9*H*-2-fluorenyl)-5-(substituted phenyl)-4,5-dihydro isoxazoles (compounds 19-27) are presented in Table 4. The study of antifungal activities of all isoxazoles against *Candida albicans* showed that compounds 23, 26 and 27 are most effective, with 20 mm zones of inhibition at 250 μg/mL per disc [33], while isoxazoles 20 and 25 are moderately active with 13-19 mm zones of inhibition and compounds 19, 20 and 22 are active with an 8-12 mm zone of inhibition. The compound containing a 4-hydroxy substituent 24 was inactive against *C. albicans.* Isoxazole derivatives 20, 26 and 27 are more effective against Penicillium species relative to compounds 21-23 and 25. The oxazoles 19 and 24 were inactive against the *Penicillium sp.* fungal strain. The zone of inhibition of isoxazoles 20, 23 and 25-27 are most effective against *Aspergillus niger* relative to compounds 19, 21 and 22. The 4-hydroxy substituted isoxazole 24 showed little to no effectiveness with any fungal strain. The presence of amino-, chloro-, dimethylamino-, methoxy- and nitro- substituents appear to be responsible for the antimicrobial activities of isoxazoles.

Antioxidant activity

The antifungal activities of the synthesized 3-(9*H*-2-fluorenyl)-5-(substituted phenyl)-4,5-dihydro isoxazoles (compounds 19-27) were measured using the DPPH radical scavenging method[34]. The observed antioxidant activities of isoxazoles are presented in Table 4. From the Table 4, the hydroxy- and methoxy-substituted isoxazoles (compounds 24 and 25) showed significant antioxidant activity. The other isoxazoles including the parent compound showed lesser antioxidant activity.

Conclusions

Totally twenty seven 3,5-diaryl-4,5-dihydro isoxazoles including 3-(9*H*-2-fluorenyl)-5-(substituted phenyl)-4,5-dihydro isoxazole derivatives have been synthesized by fly-ash:H_2SO_4 catalyzed cyclization of *E*-chalcones and hydroxylamine hydrochloride in microwave irradiation under solvent-free conditions. The yields of the isoxazoles were greater than 90%. The antimicrobial activities of the isoxazoles (compounds 19-27) have been evaluated using Bauer-Kirby methods. The chloro substituted compound shows significant antibacterial activity against all bacterial strains with 20-24 mm of zones of inhibition. Isoxazole derivatives containing amino, methoxy substituents showed greater activity with 20-24 mm zones of inhibition against *E. coli, S. aureus, K. pneumoniae* and *P. aeruginosa* bacterial strains. The parent, amino, hydroxy, methoxy and dimethylamino-substituted compounds were active against all bacterial strains, with 19-19 mm zones of inhibition. The parent, nitro, hydroxy and methoxy substituted isoxazoles were moderately active with 8-12 mm zones of inhibition against *E-coli, S. aureus,K. pneumoniae* and *E. faecalis* bacterial strains. The amino, dimethylamino, methoxy and nitro substituted isoxazoles were more active against *C. albicans*, *Penicillium* sp. and *A. niger* fungal species. The parent compound showed antifungal activity only against the *A. niger* fungal strain. The chloro substituted isoxazoles shows antifungal activity against *C. albicans* and *Penicillium* sp. fungal strains with two fungal colonies. The amino, chloro, dimethylamino, hydroxy, methoxy and nitro substituents of the isoxazoles have good antimicrobial activities. Antioxidant

activities of the isoxazoles (compounds 19-27) were measured by a DPPH radical scavenging method; compounds containing hydroxyl and methoxy substituents showed antioxidant activity.

References

1. P. G. Baraldi, A. Barco, S. Benetti, G. P. Pollini, D. Simoni; Synthesis., 857 (1987). P. A. Jacobi, K. Lee; J. Am. Chem. Soc., 119, 3409 (1997).

2. Denmark SE, Kallemeyn JM (2005) Synthesis of 3,4,5-trisubstituted isoxazoles via sequential [3 + 2] cycloaddition/silicon-based cross-coupling reactions. J Org Chem 70: 2839-2842.

3. Kan Unk O H, Adachi I, Kido R, Hirose K (1967) Isoxazoles. XVIII. Synthesis and Pharmacological Properties of 5-Aminoalkyl- and 3-Aminoalkylisoxazoles and Related Derivatives. J Med Chem 10: 411-418.

4. Pineiro M, V. D TM. Pinho-e-Melo (2009) Microwave-Assisted 1,3-Dipolar Cycloaddition: an Eco-Friendly Approach to Five-Membered Heterocycles. Eur J Org Chem 2009: 5287-5307.

5. Chakraborty B, Sharma P. K, Rai N, Devi Sharma C (2012) Solvent-free one-pot 1,3-Dipolar cycloaddition reactions of dihydropyran derived nitrone. J Chem Sci 124: 679-685.

6. Mabrour M, Bougrin K, Benhida R, Loupy A, Sohfiaoui M (2007) Tetrahedron Lett 48: 443.

7. Duan P, Yang Y, Ben R, Yan Y, Dai L, Hong Het (2014); Chem. Sci., 4, 1674.

8. Martorana A1, Piccionello AP, Buscemi S, Giorgi G, Pace A (2011) Synthesis of 4(5)-phenacyl-imidazoles from isoxazole side-chain rearrangements. Org Biomol Chem 9: 491-496.

9. K. Suneel Kumar, K. Tatendra Reddy, A. Vamsikanth, G. Omprakash, P. K. Dubey; Der Pharm. Chem., 3, 113 (2011).

10. S. S. Panda, P. V. R. Chowdary, B. S. Jayashree; Indian J. Pharm. Sci., 71, 684 (2009).

11. V. D. Joshi, M. D. Kshirsagar, S. Singhal; J. Chem. Pharm. Res., 4, 3234 (2012).

12. V. V. Kachhadia, M. R. Patel, H. S. Joshi; J. Sci. Islamic Republic Iran., 15, 47 (2004).

13. R. Janaki Rama Rao, A. K. S. Bhujanga Rao, N. Sreenivas, B. Suneel Kumar, Y. L. N. Murthy; J. Korean Chem. Soc., 55, 243(2011).

14. M. Shailaja, A. Manjula, B. Vital Rao; Indian J. Chem., 50B, 214 (2011).

15. N. Sharath, H. S. Bhojya Naik, B. Vinay Kumar, J. Hosekeri; Der Pharm. Sinica., 3, 254 (2012).

16. K. Ajay Kumar, P. Jayaroopa; Int. J. Pharm. Chem. Biol. Sci., 3, 294 (2013).

17. M. T. Shreenivas, B. E. Kumaraswamy, J. G. Manjunathan, U. Chandra, G. R. Srinivasa, B. S. Sherigara; Der Pharm. Chem., 3, 224 (2011).

18. K. Ajay Kumar, D. M. Lokeswari, G. Vasanth Kumar; Int. J. Pharm. Sci. Drug Res., 4, 236 (2012).

19. K. Madhavi, K. Bharathi, K.V.S.R.G. Prasad; Res. J. Pharm. Biol. Chem. Sci., 1, 1073 (2010).

20. F. A. Omar, A. A. A. Hafez, M. S. Ahmed; Bull. Pharm. Sci. Assiut Univ. 27, 171(2004).

21. Rahman MU1, Rathore A1, Siddiqui AA1, Parveen G2, Shahar Yar M1 (2014) Synthesis and antihypertensive screening of new derivatives of quinazolines linked with isoxazole. Biomed Res Int 2014: 739056.

22. R. T. Parihar, S. P. Rathod, P. R. Rajput; Rasayan J. Chem. 4, 660 (2011).

23. MaczyÅ„ski M, Ryng S, Artym J, Kocieba M, Zimecki M, et al. (2014) New lead structures in the isoxazole system: relationship between quantum chemical parameters and immunological activity. Acta Pol Pharm 71: 71-83.

24. Singh S1, Avor KS, Pouw B, Seale TW, Basmadjian GP (1999) Design and synthesis of isoxazole containing bioisosteres of epibatidine as potent nicotinic acetylcholine receptor agonists. Chem Pharm Bull (Tokyo) 47: 1501-1505.

25. C. Gopinath, N. Rama Rao, K. Lakshmi, N. Lakshmi Prasanthi, C. Vijaya baskar, M. K. Prahllad, R. Ramakrishna; J. Global Trend Pharm. Sci., 1, 26 (2011).

26. S. B. Garud, L. P. Shinde; Int. J. Res. Pharm Chem., 4, 46 (2014).

27. Matei, I. Chiorescu, S. Ionescu, E. Merisor, M. Hillebrand; Rev. Roum. Chim., 55, 1039 (2010).

28. Bauer AW, Kirby WM, Sherris JC, Turck M (1966) Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 45: 493-496.

29. G. Vanangamudi, M. Subramanian, G. Thirunarayanan; Arabian J. Chem., (2013) DOI: 10.1016/j.arabjc.2013.03.006.

30. Thirunarayanan G1, Mayavel P, Thirumurthy K (2012) Fly-ash:Hâ,,SOâ,,, catalyzed solvent free efficient synthesis of some aryl chalcones under microwave irradiation. Spectrochim Acta A Mol Biomol Spectrosc 91: 18-22.

31. P. Sudhir, C. Rajashree, B. Ashok; E-J. Chem., 9, 1760 (2012).

32. P. Janaki, K. G. Sekar, G. Thirunarayanan, Org. Chem.: An Indian Journal., 9(1), 68 (2013).

33. G. Thirunarayanan; Ovidius Univ. Annals Chem., 27(1), (2016), In Press; doi: 10.1515/ auoc-2016-0003.