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Green Synthesis of Isoxazoline Derivatives Using Microwave Irradiation and Their Antifungal Activity

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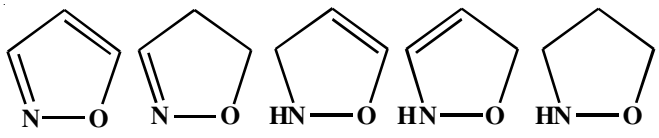
Microwave irradiation method was used for synthesis of isoxazolines. Claisen Schmidt reaction of different aromatic aldehydes with acetanilide gave acrylamides (**1a-1h**) which on further reaction with hydroxylamine hydrochloride (in the presence of sodium hydroxide) afforded isoxazolines (**2a-2h**). Physical data of all the synthesized compounds were recorded. Isoxazolines were characterized by their IR and ^{1}H NMR spectra. All the synthesized isoxazolines were screened for their antifungal activity against fungi namely *Drechslera maydis* and *Rhizoctonia solani* isolated from maize. Isoxazolines having chloro substitution on benzene ring found to be most effective followed by fluoro and nitro substituted compounds. None of the compound was registered as effective as Bavistin.

Keywords: Acrylamides, Antifungal activity, Isoxazolines, ED_{50}.

INTRODUCTION

Isoxazole is an azole (**I**) containing oxygen and nitrogen atoms at 1, 2 positions, its partially saturated analogs are isoxazolines (**II-IV**) and completely saturated analog of isoxazoline (**V**).



I **II** **III** **IV** **V**

Isoxazole nucleus is a structural moiety found in natural products such as ibotenic acid and in synthetic compounds with vital medicinal value. Isoxazoles have illustrious history *e.g.*, their chemistry is associated with Ludwig Claisen, who first recognized the cyclic structure of 3-methyl-5-phenyl-isoxazole in 1888 and was shown to possess typical properties of an aromatic system under certain reaction conditions particularly in basic media, it is highly labile. A very significant contribution to the development of isoxazole chemistry came between 1930-1946 from Quilico's studies on the synthesis of ring system from nitrile oxides and unsaturated compounds.

Isoxazoles also form the basis for a number of drugs, including the COX-2 inhibitor valdecoxib (bextra), a derivative of furoxan, which is a nitric oxide donor. Isoxazoline derivatives controlled *B. cineria* on cucumbers [1], antiviral properties against herpes type 2 viruses [2] and also possess anti-influenza virus activity [3]. Penicillin derivatives containing isoxazole ring were found to be antibacterial [4].

Isoxazoline derivatives possess antidiabetic, diuretic, analgesic, anthelimintic and hypolipaemic activity. There are many interesting biological activities exhibited by these compounds. Isoxazoline derivatives are used as corrosion inhibitors for fuels and lubricants [5]. These derivatives also show a good potency in animal models of thrombosis [6].

Microwave heating has emerged as a powerful technique to promote a variety of chemical reactions [7-11]. Microwave reactions under solvent-free conditions were attractive in offering reduced pollution and offer low cost technique together with simplicity in processing and handling [12-14]. Microwave-assisted synthesis includes virtually all types of chemical reactions such as additions, cycloadditions, substitutions, eliminations, fragmentations, *etc*. Recently reported studies on the microwave irradiation for the synthesis of heterocyclic compounds revealed that it is safe rapid, economic and convenient, ecofriendly method. It can be termed as e-chemistry because it is easy, effective, economical and eco-friendly and is believed to be a step towards green chemistry. Keeping in view the above facts, the present study was carried out to synthesize isoxazoline derivatives using microwave and their evaluation for antifungal activity.

EXPERIMENTAL

All the recorded melting points were determined in open capillaries and are uncorrected. Structures of compounds were confirmed by routine spectrometric analysis. IR and ^{1}H NMR spectra were scanned at Sophisticated Analytical Instrumentation Facility (SAIF), Central Instrument Laboratory (CIL), Panjab University, Chandigarh. The IR spectra of compounds were recorded using KBr discs on Perkin-Elmer FTIR spectrophotometer. ^{1}H NMR spectra were recorded on a Bruker Avance II 400 MHz instrument using TMS as an internal standard. The chemical shifts were expressed in δ (ppm) values.

General procedure forsynthesis of isoxazolines (2a-2h): Equimolar amounts of already prepared acrylamides [15] (**1a-1h**) (0.005 mol), hydroxylamine hydrochloride (0.005 mol) in ethanol (30 mL) were taken in an Erlenmeyer flask of 100 mL. 2-3 drops of NaOH solution (30 %) was added. The reaction mixture was mixed thoroughly and irradiated at 720 W with intermitted irradiation for 30 s. The progress of reaction was checked with TLC. On completion of reaction resultant mass was poured into ice cold water with stirring. The solid obtained was filtered, washed and recrystallized from ethanol. Physical data of all the synthesized compounds is given in Table-1.

TABLE-1
PHYSICAL PARAMETERS OF ISOXAZOLINES

Compd.	m.f.	Colour	Yield (%)	R_f	m.p. (°C)
2a	$C_{15}H_{14}N_2O$	White	50	0.61	111-113
2b	$C_{15}H_{13}N_2OCl$	Off white	68	0.59	115-116
2c	$C_{15}H_{13}N_2OCl$	Yellow	70	0.58	90-91
2d	$C_{15}H_{13}N_2OF$	Yellow	66	0.56	94-95
2e	$C_{15}H_{14}N_2O_2$	Brown	71	0.54	–
2f	$C_{15}H_{14}N_2O_2$	Light brown	70	0.55	106-108
2g	$C_{15}H_{15}N_3O_3$	Orange	58	0.54	97-99
2h	$C_{15}H_{13}N_3O_3$	Yellow	63	0.65	116-118

Testing of antifungal activity: Synthesized isoxazolines (**2a-2h**) were evaluated for their *in vitro* antifungal activity against *D. maydis* and *R. solani* by poisoned food technique [16]. The per cent inhibition and ED_{50} of synthesized compounds against both the test fungi was determined.

N-5-Diphenyl-4,5-dihydro isoxazol-3-amine (2a): Yield 50 %, m.p.: 111-113 °C. IR (KBr, ν_{max}, cm^{-1}): 3360 (N-H str.), 3038 (Ar C-H str.), 1597 (C=N str.), 1263 (C-O str.), 757 (N-H bending). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm) 2.57-2.64 (dd, 1H, CH_2, *J* = 18.3, 4.3 Hz), 3.33-3.40 (dd, 1H, CH_2, *J* = 18.5, 11.4 Hz), 4.22-4.26 (dd, 1H,CH, *J* = 11.6, 4.4 Hz), 7.49-7.89 (m, 10H, Ar-H), 10.25 (s, 1H, NH).

5-(2-Chlorophenyl)-N-phenyl-4,5-dihydro isoxazol-3-amine (2b): Yield 68 %, m.p.: 115-116 °C. IR (KBr, ν_{max}, cm^{-1}): 3320 (N-H str.), 3020 (Ar C-H str.), 1585 (C=N str.), 1260 (C-O str.), 1025 (C-Cl str.), 760 (N-H bending). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm) 2.57-2.63 (dd, 1H, CH_2, *J* = 16.3, 5.3 Hz), 3.38-3.44 (dd, 1H, CH_2, *J* = 16.3, 11.4 Hz), 5.23-5.27 (dd, 1H, CH, *J* = 11.6, 4.4 Hz), 7.19-7.89 (m, 9H, Ar-H), 10.82 (s, 1H, NH).

5-(4-Chlorophenyl)-N-phenyl-4,5-dihydro isoxazol-3-amine (2c): Yield 70 %, m.p.: 90-91 °C. IR (KBr, ν_{max}, cm^{-1}): 3350 (N-H str.), 3056 (Ar C-H str.), 1605 (C=N str.), 1256 (C-O str.), 1053 (C-Cl str.), 745 (N-H bending). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm) 3.77-3.83 (dd, 1H, CH_2, *J* = 17.3, 6.3 Hz), 4.03-4.07 (dd, 1H, CH_2, *J* = 16.5, 7.4 Hz), 5.04-5.09 (dd, 1H, CH, *J* = 16.6, 6.2 Hz)., 7.68-7.8.24 (m, 9H, Ar-H), 9.92 (s, 1H, NH).

5-(4-Fluorophenyl)-N-phenyl-4,5-dihydro isoxazol-3-amine (2d): Yield 66 %, m.p.: 94-95 °C. IR (KBr, ν_{max}, cm^{-1}): 3349 (N-H str.), 3050 (Ar C-H str.), 1594 (C=N str.), 1239 (C-O str.), 1050 (C-F str.), 768 (N-H bending).^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm) 3.77-3.83 (dd, 1H, CH_2, *J* = 17.3, 6.3 Hz), 4.13-4.20 (dd, 1H, CH_2, *J* = 17.5, 7.4 Hz), 5.14-5.19 (dd, 1H, CH, *J* = 16.6, 6.2 Hz), 7.49-8.29 (m, 9H, Ar-H), 9.72 (s, 1H, NH).

2-[3-(Phenylamino)-4,5-dihydro isoxazol-5-yl]phenol (2e): Yield 71 %. IR (KBr, ν_{max}, cm^{-1}): 3399 (broad N-H, O-H str.), 1594 (C=N str.), 1393 (C-N str.), 1268 (C-O str.), 759 (N-H bending). ^{1}H NMR (400 MHz, D_2O, δ, ppm) 2.58-2.63 (dd, 1H, CH_2, *J* = 18.3, 4.3 Hz), 3.31-3.39 (dd, 1H, CH_2, *J* = 19.7, 12.6 Hz), 5.15-5.20 (dd, 1H, CH, *J* = 11.5, 4.4 Hz), 7.61-8.15 (m, 9H, Ar-H), 10.24 (s, 1H, NH), 10.42 (s, 1H, O-H).

4-[3-(Phenylamino)-4,5-dihydro isoxazol-5-yl]phenol (2f): Yield 70 %, m.p.: 106-108 °C. IR (KBr, ν_{max}, cm^{-1}): 3440 (broad N-H, O-H str.),1592 (C=N str.), 1250 (C-O str.), 770 (N-H bending). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm) 3.75-3.79 (dd, 1H, CH_2, *J* = 16.4, 6.3 Hz), 4.03-4.08 (dd, 1H, CH_2, *J* = 16.7, 7.4 Hz), 5.04-5.09 (dd, 1H, CH, *J* = 16.6, 6.2 Hz), 7.68-8.24 (m, 9H, Ar-H), 9.92 (s, 1H, O-H),10.55 (s, 1H, NH).

5-(2-Nitrophenyl)-N-phenyl-4,5-dihydro isoxazol-3-amine (2g): Yield 58 %, m.p.: 97-99 °C. IR (KBr, ν_{max}, cm^{-1}): 3340 (N-H, str.), 3070 (Ar C-H str.), 1593 (C=N str.), 1510 (N=O str.), 1255 (C-O str.), 765 (N-H bending). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm) 2.77-2.82 (dd, 1H, CH_2, *J* = 17.3, 6.3 Hz), 3.03-3.07 (dd, 1H, CH_2, *J* = 16.9, 7.4 Hz), 5.12-5.17 (dd, 1H, CH, *J* = 12.6, 6.2 Hz), 7.44-8.04 (m, 9H, Ar-H), 9.52 (s, 1H, NH).

5-(4-Nitrophenyl)-N-phenyl-4,5-dihydro isoxazol-3-amine (2h): Yield 63 %, m.p.: 116-118 °C. IR (KBr, ν_{max}, cm^{-1}): 3380 (N-H, str.), 3028 (Ar C-H stretching), 1596 (C=N str.), 1550 (N=O str.), 1253 (C-O str.), 785 (N-H bending). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm) 3.77-3.81 (dd, 1H, CH_2, *J* = 17.1, 6.3 Hz), 4.15-4.19 (dd, 1H, CH_2, *J* = 16.5, 7.4 Hz), 5.04-5.08 (dd, 1H, CH, *J* = 16.6, 6.2 Hz), 7.68-8.24 (m, 9H, Ar-H), 10.52 (s, 1H, NH).

RESULTS AND DISCUSSION

Isoxazolines (**2a-2h**) were prepared by reaction of synthesized acrylamides with hydroxylamine hydrochloride using microwave method. The reactants were irradiated for 3-4 min to form the isoxazolines as shown in Fig. 1. Synthesis of isoxazolines was confirmed by their characteristics peaks in IR and ^{1}H NMR spectra. Three double doublets in the ^{1}H NMR spectra confirmed the formation isoxazoline ring system. IR spectral data provided further confirmation to the formation of isoxazoline ring. The physical parameters (colour, yield, melting point, R_f values) of synthesized compounds were determined and are presented in Table-1.

Infrared: Infrared spectral data revealed the formation of isoxazolines. N-H, C=N and C-O stretching vibrations were

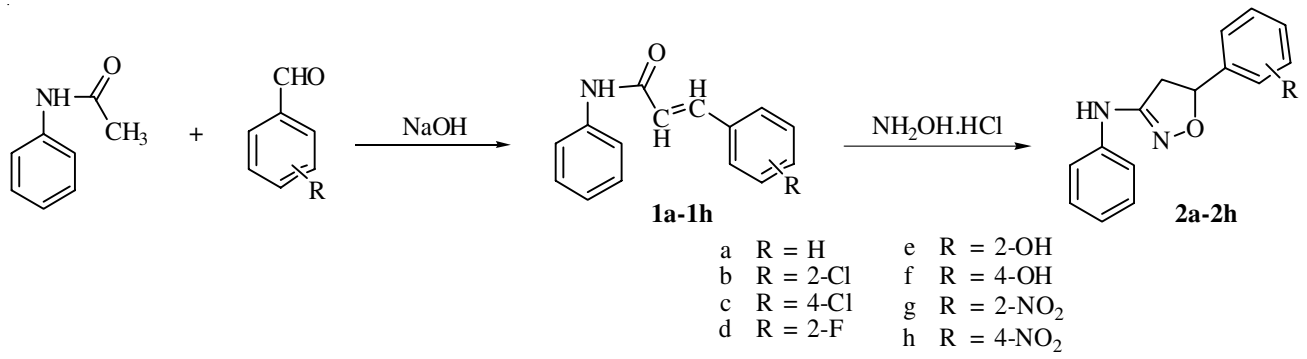
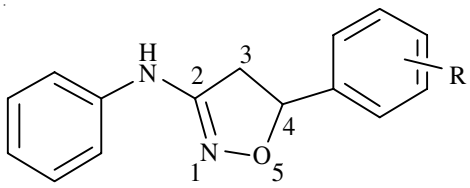


Fig. 1. Scheme for the preparation of acrylamide and isoxazolines

also observed in range of 3440-3320, 1605-1588 and 1268-1239 cm^{-1}, respectively. The characteristic >C=O stretching vibration of acrylamides was absent in IR spectra of isoxazoline which again assured the ring formation. Band for O-H stretching was observed for compounds **2e** and **2f** having hydroxy group on aromatic ring.

^{1}H NMR: The formation of isoxazolines was further confirmed by ^{1}H NMR spectrum. The two hydrogens on C-3 of isoxazoline ring (Fig. 2) were not equiva-lent. Both of these protons were observed as double doublets in range of 2.57-3.83 and 3.03-4.20 ppm. Proton at C-4 of isoxazoline also appeared as double doublet in the range of 4.22-5.27 ppm. Aromatic protons appeared as multiplet in range of 7.19-8.29 ppm. A singlet corresponding to -NH group was obtained in the range of 9.55-10.82 ppm. More confir-mation to the formation of isoxazolines was provided by coupling constant values of double doublets.



R = H, Cl, F, OH, NO_2

Fig. 2. Structure of isoxazoline ring

Antifungal activity: Isoxazolines (**2a-2h**) were screened *in vitro* for their antifungal activity against *Drechslera maydis* and *Rhizoctonia solani* by poisoned food technique. The results are expressed in ED_{50} values *i.e.* the effective dose at which 50 % inhibition has occurred. All the compounds showed moderate antifungal potential against test fungi.

Antifungal activity of isoxazolines against *D. maydis*: The data presented in Table-2 given below exhibited that all the isoxazolines were active against *D. maydis* at 50 µg/mL with inhibition ranging from 3.27 to 19.67 % but less than bavistin (48.56 %). Isoxazoline (**2c**) having chloro group at *para* position of benzene ring showed maximum activity *i.e.* 54.26 % inhibition at 1000 µg/mL which was significantly higher than other tested compounds. Isoxazoline having no substitution on benzene ring exhibited lowest per cent inhibition at all the concentrations. It was followed by compounds having hydroxyl group at *ortho* and *para* position of benzene ring, respectively. Bavistin recorded to have highest per cent inhibition at all the test concentration than all other isoxazolines.

TABLE-2
EFFECT OF DIFFERENT ISOXAZOLINES AT DIFFERENT CONCENTRATIONS (µg/mL) ON THE GROWTH OF *D. maydis*

Compd.	Inhibition (%)				
	1000	500	250	100	50
2a	26.2	18.03	9.83	6.55	3.27
2b	51.36	40.56	30.25	20.56	10.56
2c	54.26	39.34	29.50	22.95	17.31
2d	45.96	35.63	29.5	22.95	19.67
2e	29.5	26.22	21.31	14.75	9.83
2f	31.14	26.22	19.67	11.47	4.91
2g	34.76	28.65	20.85	13.11	6.55
2h	35.65	29.36	24.23	17.53	9.83
Bavistin	85.97	76.23	68.65	60.63	48.56
CD (p = 0.05)	0.007	0.005	0.006	NS	0.003

ED_{50} of only two compounds were found to be less than 1000 mg/mL against *D. maydis*as shown in Table-3. Isoxazolines (**2b**) and (**2c**) having chloro group at *ortho* and *para* position on benzene ring showed better control as compared to other tested compounds. ED_{50} of (**2b**) and (**2c**) were 945 and 863 mg/mL, respectively while all other compounds had ED_{50} value more than 1000 mg/mL.

TABLE-3
ED_{50} OF DIFFERENT ISOXAZOLINES AGAINST *D. maydis*

Compd.	ED_{50} (µg/mL)	Compd.	ED_{50} (µg/mL)
2a	–	**2f**	–
2b	945	**2g**	–
2c	863	**2h**	–
2d	–	Bavistin	60
2e	–	–	–

Antifungal activity of isoxazolines against *R. solani*: The data presented in Table-4 exhibited that all the compounds except compound having hydroxyl group at *ortho* position were active against *R. solani* at 100 µg/mL with inhibition ranging from 1.63 to 31.14 %. Compound having chloro group at *para* position of benzene ring showed maximum inhibition percentage of 86.80 at 1000 µg/mL, which was significantly higher than other tested compounds. Isoxazolines were found to be moderately effective against *R. solani* but none of the compound was found effective as compared to the standard fungicide bavistin (ED_{50} 5 mg/mL).

TABLE-4
EFFECT OF DIFFERENT ISOXAZOLINES AT DIFFERENT CONCENTRATIONS (µg/mL) ON THE GROWTH OF *R. solani*

Compd.	Inhibition (%)				
	1000	500	250	100	50
2a	27.86	18.19	13.27	11.63	0
2b	68.85	57.37	34.42	11.47	1.63
2c	86.8	68.85	45.90	24.59	14.75
2d	63.93	55.73	40.98	31.14	21.31
2e	13.27	11.63	7.63	0	0
2f	19.83	14.91	8.27	1.63	0
2g	54.09	34.42	22.95	19.65	16.39
2h	59.01	40.98	29.50	21.31	18.03
Bavistin	100	100	100	100	100
CD (p = 0.05)	0.003	0.004	0.005	NS	0.003

ED_{50} of compounds **2b**, **2c**, **2d**, **2g** and **2h** was found to be less than 1000 mg/mL against *D. maydis* as shown in Table-5. Isoxazolines having *ortho* chloro group (**2b**), *para* chloro group (**2c**) and *ortho* fluoro group (**2d**) on benzene ring showed better control as compared to other tested compounds while all other compounds had ED_{50} value more than 1000 mg/mL.

TABLE-5
ED_{50} OF DIFFERENT ISOXAZOLINES AGAINST *R. solani*

Compd.	ED_{50} (µg/mL)	Compd.	ED_{50} (µg/mL)
2a	–	**2f**	–
2b	390	**2g**	909
2c	285	**2h**	773
2d	375	Bavistin	5
2e	–	–	–

Conclusion

Reaction of different acrylamides with hydroxylamine hydrochloride under microwave irradiations yielded isoxazoline in 3-4 min. Chloro substituted isoxazolines were found to be more effective than other compounds. Further *para* chloro substituted isoxazoline was observed to have more activity than *ortho* chloro substituted compound. But all the compounds showed less antifungal activity as compared to standard bavistin.

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