Synthesis & Evaluation of isoxazole for their antimicrobial activity

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Abstract

Isoxazole is five membered heterocyclic ring having a broad spectrum of pharmacological activities like anti-tubercular, anti-cancer, anti-bacterial, anti-fungal, anti-HIV, anti-inflammatory and anti-hypertensive activities.

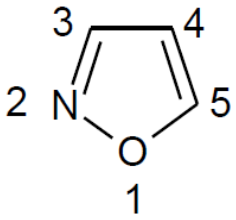
In the present research work we reported the synthesis of some novel isoxazoles by using various different substituted chalcones and screened for their anti-microbial activity.

Keyword: Isoxazole, Anti-microbial activity.

Introduction

Nitrogen containing heterocycles with an oxygen atom are considered as an important class of compounds in medicinal chemistry because of their diversified biological applications. The exploitation of a simple molecule with different functionalities for the synthesis of heterocycles is a worthwhile contribution in the chemistry of heterocycles.

Isoxazole is a five membered heterocyclic compound containing oxygen and nitrogen atoms in the 1, 2 positions, its partially saturated analogs are called isoxazolines and completely saturated analog is isoxazolidine.



Isoxazoles are an important class of heterocycles, which are largely employed in the area of pharmaceuticals and therapeutics such as insecticidal, antibacterial, antibiotic, antitumour, antifungal, antituberculosis, anticancer and ulcerogenic. Isoxazole derivatives are used in the market as COX-2 inhibitor and anti-inflammatory drugs.

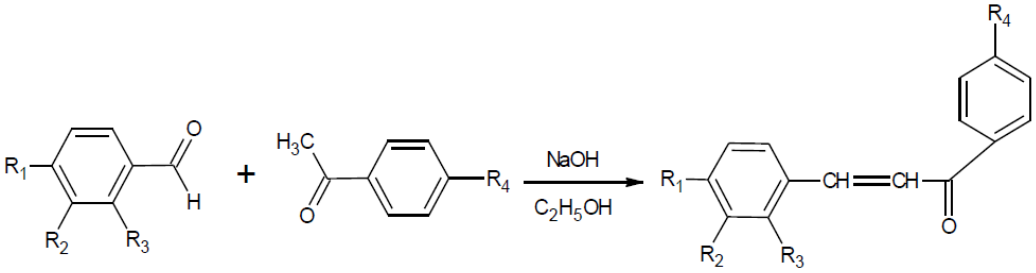
Although isoxazole derivatives have been known for more than 80 years, the investigation of their chemistry commended rather slowly. Earlier studies were mainly devoted to the development of synthetic methods. Recently the attention was focused on the investigation of chemical properties and in particular on the peculiarities of the behaviour of isoxazole derivatives and the elucidation of their physicochemical characteristics. This enabled new datas to be obtained that were considerable importance.

Materials and Methods

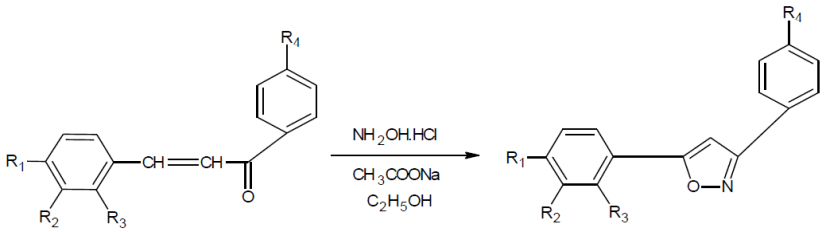
All the melting points were determined on Micro-controller based melting point apparatus CL 725/726 and were uncorrected. Chloro and nitro benzaldehydes were purchased from Techno chemicals, Bangalore. Other chemicals like hydroxyl amine hydrochloride & sodium acetate were purchased from S.D. Fine chemicals, Bangalore. Silica gel G plates (3x8cm) were used for TLC and spots were located by UV or in iodine chamber. The IR spectra (KBr) were determined on FTIR 8400S, SHIMADZU Spectrometer and the values were expressed in cm-1 1H-NMR were recorded in either CDCl3 or DMSO-d6 solvents using TMS as an internal reference standard at IIT Chennai and II Sc Bangalore.

General procedure for synthesis of chalcones and cyclization

Step I: Equimolar quantities of different substituted aromatic benzaldehyde (0.01mol) and substituted aromatic acetophenones (0.01mol) were dissolved in 25 mL of alcohol. Sodium hydroxide solution (0.02mol) was added slowly and the mixture stirred for 12 hr. until the entire mixture becomes very cloud. Then the mixture was poured slowly into 400 mL. of water with constant stirring and kept refrigerator for 24hr. Then precipitate obtained was filtered. Washed and recrystallised from ethanol.



Step II: 0.015 mol of chalcone, 0.015mol of hydroxyl ammonium hydrochloride and sodium acetate 0.015mol in 25 mL of ethanol was refluxed for 6hr. The mixture was concentrated by distilling out the solvent under reduced pressure and poured into ice cold water. The precipitate obtained was filtered, washed and recrystllized from acetone.



N1: 1-(4-nitrophenyl)-3-(4-methoxyphenyl)prop-2-en-1-one
Yellow Colour crystals, **yield:** 65%, **MP:** 155ºC, **FTIR (KBr):** 1657 (C=C), 1573 (C=N), 2855 (C-H), 1031 (C-O) 1440 (R-NO2).**^{1}H NMR (DMSO):** 3.8-4 (s, CH3), 6.7-8.3(m, Ar-H, 8H, CH, 1H). **MS:** m/z (%) 296.09 (57%)[M^+]. [Found: C.64.86, H.4.08, N.9.45, O.21.60 $C_{16}H_{12}N_2O_4$ requires C.64.83, H.4.03, N. 9.35%].

N2: 1-(4-nitrophenyl)-3-(4nitrophenyl)prop-2-en-1-one
Brown Colour crystals, **yield:** 60% , **MP:** 185ºC, **FTIR (KBr):** 1595 (C=C), 1597 (C=N), 1711.33 (C-O), 1456 (R-NO2).[Found: C.57.88,H.2.91, N.13.50, O.25.70 $C_{15}H_9N_2O_5$ requires C.57.82,H.2.88,N.13.45%].

N3: 1-(4-nitrophenyl)-3-(3-nitrophenyl)prop-2-en-1-one
Colourless crystal, **yield:** 62%, **MP:** 160ºC, **FTIR (KBr):** 1649(C=C),1593(C=N),2967 (C-H),1100(C-O) 1374 (R-NO2).[Found:C.52.20,H.2.63,N.8.12,O.13.91 $C_{15}H_9N_2O_5$ requires C.52.18,H.2.60,N.8.10%].

N4: 1-(4-nitrophenyl)-3-(4-bromophenyl)prop-2-en-1-one
Pale Brown crystal, **yield:** 50%, **MP:** 169ºC, **FTIR (KBr):** 1597(C=C),1527(C=N),3191 (C-H),1101(C-O), 1494 (R-NO2),692 (C-Br).[Found: C.52.20,H.2.63,Br.23.15,N.8.12,O.13.91 $C_{15}H_9N_2O_3Br$ requires C.52.22,H.2.59,N.8.14%].

N5: 1-(4-nitrophenyl)-3-(4-chlorophenyl)prop-2-en-1-one
Brown Colour crystal, **yield:** 60%, **MP:** 156ºC,**FTIR (KBr):** 1518(C=C),1622(C=N),3150 (C-H),1015(C-O), 1447 (R-NO2),693(C-Cl).[Found: C.59.91,H.3.02,Cl.11.29,N.9.32,O.15.96 $C_{15}H_9N_2O_3Cl$ requires C.59.89,H.3.09,N.9.34%].

N6: 1-(4-nitrophenyl)-1-(4-Phenol)prop-2-en-1-one
Brown Colour crystal, **yield:** 62%, **MP:** 155ºC, **FTIR (KBr):** 1515(C=C),1688(C=N),2954 (C-H),1009(C-O),3254(C-OH).[Found: C.63.83,H.3.57, N.9.92,O.22.67 $C_{15}H_9N_2O_3$ requires C.63.80,H.3.54,N.9.94%].

N7: 1-(4-nitrophenyl)-3-(3-phenol)prop-2-en-1-one
Brown Colour crystal, **yield:** 65%, **MP:** 170ºC, **FTIR (KBr):** 1578(C=C), 1672(C=N),2927 (C-H),1100(C-O), 1390(R-NO2),3218(C-OH).[Found: C.63.83,H.3.57,N.9.92,O.22.67 $C_{15}H_{10}N_2O_3$ requires C.63.80,H.3.54,N.9.94%].

N8: 1-(4-nitrophenyl)-3-(3-methoxyphenyl)prop-2-en-1-one
Yellow Colour crystal, **yield:** 62%, **MP:** 160ºC, **FTIR (KBr):** 1494(C=C),2959(C-H),1036(C-O), 1397.[Found: C.64.86,H.4.08,N.9.45,O.21.60 $C_{16}H_{12}N_2O_4$ requires C.64.84,H.4.04,N.9.44%].

N9: 1-(4-nitrophenyl)-3-(4-(dimethylamino)phenyl)prop- 2-en-1-one
Brown Colour crystal, **yield:** 72%, **MP:** 178ºC, **FTIR (KBr):** 1519(C=C),1647(C=N),2911.19 (C-H),1066(C-O),1519(R-NO2). **^{1}H NMR (DMSO):** 2.77(s, CH3), 6.7-8.4(m, Ar-H,CH,1H).

MS:m/z(%)309(57%)[M^+].[Found:C.68.34,H.5.06,N.9.38,O.5.36$C_{17}H_{15}N_3O_3$requires C.68.30,H.5.02,N.9.34%].

C1: 3-(4-chlorophenyl)-5-(4-methoxyphenyl)isoxazole
Yellow Colour crystal, **yield:** 50%, **MP:** 110ºC, **FTIR (KBr):** 1509(C=C),1654(C=N),2965 (C-H),1029(C-O),739(C-Cl).[Found:C.67.26,H.4.23,N.4.90,O.11.20,Cl.12.41$C_{16}H_{12}NO_2Cl$requires C.67.30,H.4.22,N.4.89%].

C2: 3-(4-chlorophenyl)-5-(4-nitrophenyl)isoxazole
Colourless crystal, **yield:** 60%, **MP:** 90ºC, **FTIR (KBr):** 1519(C=C),1637(C=N),3295 (Ar-C-H),1012(C-O),750(C-Cl).
[Found:C.59.91,H.3.02,N.9.32,O.15.96,Cl.11.79$C_{15}H_9N_2O_5Cl$requires C.59.89,H.3.05,N.9.30%].

C3: 3-(4-chlorophenyl)-5-(3-nitrophenyl)isoxazole
C4: 5-(4-bromophenyl)-3-(4chlorophenyl)isoxazole
C5: 3,5-bis(4-chlorophenyl)isoxazole
C6: 4-[3-(4-chlorophenyl)isoxazol-5-yl]phenol
C7: 3-[3-(4-chlorophenyl)isoxazol-5-yl]phenol
C8: 3-(4-chlorophenyl)-5-(3-methoxyphenyl)isoxazole
C9: 4-[3-(4-chlorophenyl)isoxazol-5-yl]-*N*,*N*-dimethylaniline

Methodology

Equimolar quantities of aromatic aldehydes and aromatic acetophenones were dissolved in 25 mL of alcohol. Sodium hydroxide solution (0.02 mol) was added slowly and the mixture stirred for 12 hr. until the entire mixture becomes very cloud. Then the mixture was poured slowly into 400 mL of water with a constant stirring and kept in refrigerator for 24 hr. Then precipitate obtained was filtered, washed and recrystallized from ethanol.

A mixture of chalcone hydroxylamine hydrochloride (0.02 mol) and sodium acetate (0.02 mol) in ethanol (25 mL) was refluxed for 6 hr. The mixture was concentrated by distilling out the solvent under reduced pressure and poured into ice water. The precipitate obtained was filtered, washed and recrystallized from acetone.

Experimental Section: All the melting points were determined on Micro-controller based melting point apparatus CL 725/726 and were uncorrected. Chloro and nitro benzaldehydes were purchased from Techno chemicals, Bangalore. Other chemicals like hydroxyl amine hydrochloride & sodium acetate were purchased from S.D. Fine chemicals, Bangalore. Silica gel G plates (3x8cm) were used for TLC and spots were located by UV or in iodine chamber. The IR spectra (KBr) were determined on FTIR 8400S, SHIMADZU Spectrometer and the values were expressed in cm^{-1} ^{1}H-NMR were recorded in either $CDCl_3$ or DMSO-d_6 solvents using TMS as an internal reference standard at IIT Chennai and IISc Bangalore.

Biological Activity

Antibacterial activity:[21-29] The successive isoxazole derivatives were tested for antibacterial activity systematically against four different strains of bacteria (gram-positive and gram negative) by the agar cup and plate method.

Generally, the antibacterial activity of a compound is expressed in terms of its ability to inhibit the growth of bacteria in nutrient broth or agar; the bacterial inhibition can be measured by two methods: one is the serial dilution method and the other is diffusion method. The serial dilution method is very much useful for the determination of the antibacterial activity. But it is not much useful for the qualitative detection tests and also for the evaluation of a large number of compounds. Therefore, in this investigation the latter is employed. Further, the contemplated agar diffusion method is of three types: (i) Cup-plate method (disc method), (ii) Filter paper strip method, and (iii) Gradient plate method.

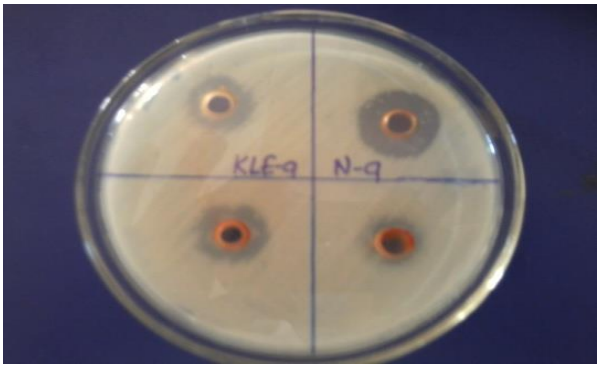
The specific method adopted in the present investigation was cup-plate method involving discs of standard diameter, the nutrient agar medium and containing standard bacterial inoculum. The test compounds were introduced into the discs and the diameters of the zones of inhibition were measured. All the derivaties were evaluated for antibacterial activity against, *Eschrichia coli*, *Pseudomonas aeruginosa*, *Klebsiella*, *Staphyllococcus aureus* following the agar diffusion method.

- The organisms were sub-cultured using nutrient agar medium. The tubes containing sterilized medium were inoculated with respective bacterial strain. Stock cultures were maintained. Bacterial inoculum was prepared by transferring a loop full of stock culture to nutrient broth (100 mL) in a clean sterilized conical flask (250 mL).
- The flasks were incubated at 37 ± 1ºC for 18 h before the experimentation. Solutions of the compounds were prepared by dissolving 10 mg of each in 1 mL DMSO.
- Reference standard for gram-positive and gram-negative bacteria were made by dissolving accurately weighed quantity of ciprofloxacin, respectively in DMSO solution, separately. The nutrient agar medium was sterilized by autoclaving at 121ºC (15 lb/sq. inch).
- The petri-plates, tubes and flasks plugged with cotton were sterilized in hot air-oven at 160ºC for an hour. Into each sterilized petri-plate (10cm diameter), about 30 ml each of molten nutrient bacteria (6 mL of inoculum to 300 mL of nutrient agar medium) was transferred, aseptically.
- The plates were left at room temperature to allow the solidification. In each plate, four wells of 6 mm

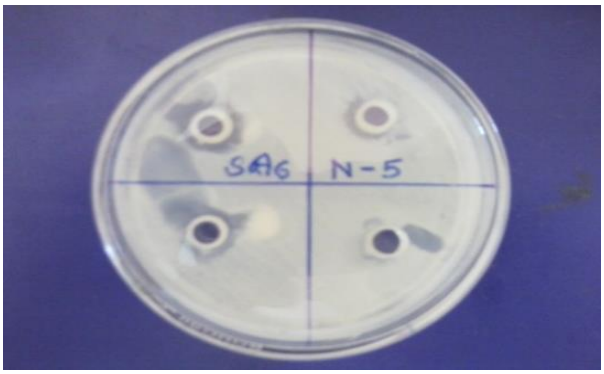
diameter were made with a sterile borer. Then, 0.1 ml of the test solution was added to the discs, aseptically and labelled, accordingly. The plates were kept undisturbed for at least 2 h at room temperature to allow diffusion of the solution properly, into nutrient 2 h. at room temperature to allow diffusion of the solution properly, into nutrient agar medium.

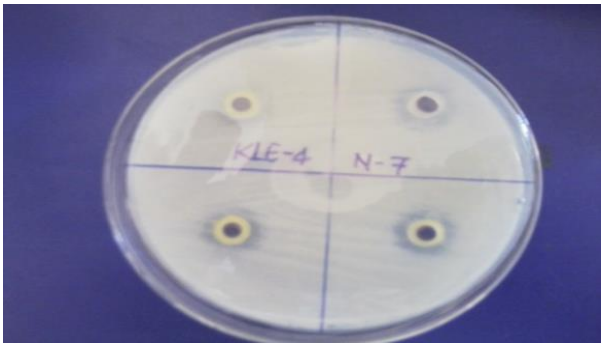
- After incubation of the plates at 37 ± 1°C for 24 h. the diameter of the zone of inhibition surrounding each of the discs was measured with the help of an 'antibiotic zone reader. All the experiments were carried out in triplicate. Simultaneously, controls were maintained employing 0.1 ml of methanol to observe the solvent effects.

Mean zone of inhibition is including disc diameter: Disc diameter is 6 mm



N_9 Klebsiella Pneumoniae C_4 Staphylococcus aureus





N_5 Staphylococcus aureus N_7 Klebsiella Pneumoniae

Table 1: Antibacterial activity of Isoxazole derivatives at different concentration by well diffusion method (values in mm)

Compound code	Mean zone of inhibition (in mm)															
	S .Aureus				E. coli				P. Auruginosa				Klebsiella			
	100 µg/ml	200 µg/ml	400 µg/ml	500 µg/ml	100 µg/ml	200 µg/ml	400 µg/ml	500 µg/ml	100 µg/ml	200 µg/ml	400 µg/ml	500 µg/ml	100 µg/ml	200 µg/ml	400 µg/ml	500 µg/ml
Standard Ciprofloxacin	8	11	14	18	26	28	30	31	30	31	31	34	26	30	32	34
N_1	-	-	-	-	8	11	13	16	-	-	-	-	9	10	12	14
N_2	-	-	-	-	-	-	-	-	-	-	-	17	9	12	16	18
N_3	8	8	10	11	-	-	-	-	-	-	-	-	-	-	-	-
N_4	-	-	-	-	-	-	-	-	-	12	14	15	8	10	12	14
N_5	7	9	11	14	-	-	-	-	-	-	-	-	10	12	14	15
N_6	-	-	-	-	-	-	-	-	7	9	11	12	8	10	11	12
N_7	-	-	-	-	-	-	-	-	-	-	-	-	9	12	15	16
N_8	5	7	7	8	-	-	-	-	-	-	-	-	-	8	11	15
N_9	-	-	-	-	9	11	13	13	9	12	14	15	11	14	16	18
C_1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C_2	8	10	13	16	-	-	-	-	7	9	10	14	-	-	-	-
C_3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C_4	9	10	12	16	-	-	-	-	-	-	-	-	10	11	13	15
C_5	5	6	6	7	-	-	-	-	-	-	-	-	6.5	7	9	11
C_6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C_7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C_8	-	-	-	-	8	10	11	12	-	-	-	-	10	14	16	18

Table 2: Lipophilicity of isoxazole derivatives compounds 1-18

Sr. No.	Compound code	$logP_{10}$
1	N_1	4.27
2	N_2	3.91
3	N_3	3.89
4	N_4	4.83
5	N_5	5.04
6	N_6	3.86
7	N_7	3.84
8	N_8	4.24
9	N_9	4.31
10	C_1	5.37
11	C_2	5.04
12	C_3	5.06
13	C_4	5.69
14	C_5	5.83
15	C_6	4.76
16	C_7	4.75
17	C_8	5.35
18	C_9	4.92

Summary and Conclusion

Isoxazole have played crucial role in the history of hetrocyclic chemistry and been extensively important pharmacophores and synthons in the field of organic chemistry owing to their versatile chemotherapeutic importance, a significant amount of research effort has been focused on these nuclei. Isoxazole is a five membered heterocyclic ring system containing oxygen and nitrogen atoms have been reported to posses anthelmintic, antibacterial, ant hyperglycemic, analgesic, anti-inflammatory, antipyretic, antiviral and antitumor properties.

In present study we fused the two moieties (aromatic substituted ketone and aromatic substituted aldehyde) to isoxazole with the view to get good pharmacological activity and less toxicity.

As expected, isoxazole derivatives exhibited both anti-bacterial activities in which some compounds are good and some are moderately active like standard employed for comparision. The antibacterial activity which has done on some gram-positive and gram negative showed that few compounds were exhibiting the antibacterial activity by observing zone of inhibition.

Further the detailed structural activity relationship studies are required along with the molecular manipulation i.e. molecular modeling may give better drugs. Molecules prepared for the biological testing do not always turn out as potential new drugs, but may be intended to serve as models for evaluation of hypothesis.

The compound N1 has Isoxazole nucleus with groups OCH_3 at position R1 and NO2 at R4 also showed enhancement in anti-bacterial activity.

In the compound N9 substitution of R1 of dimethyl amino and NO2 at R4 position of the aromatic ring also resulted in a enhancement of anti-bacterial activity. In the compound C4, C9 substitution Br and N(CH)3 at R1 position and Cl at R4also showed significant anti-bacterial activity.

Hence in the present study, the aromatic substituted ketone and aromatic substituted aldehydes when linked with isoxazole moiety showed highly potent, more specific antibacterial activity.

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