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Synthesis, Characterization and *In-Vitro* Antimicrobial Evaluation of Some Novel Isoxazoline Derivatives

Rajeev Bhimwal*[1], Anil K Sharma[2], Ankit Jain[3]

1. Mahatma Gandhi College of Pharmaceutical Sciences, Jaipur, Rajasthan, India.
2. Jaganath University, Jaipur, Rajasthan, India.
3. College of Pharmacy, Indore Professional Studies (IPS) Academy, Rajendra Nagar, Indore, (M.P.), India.

*Corresponding author: rajeevb.himwal.2005@gmail.com

ABSTRACT:

Different isoxazolines (I1-I6) were synthesized via cyclization of substituted chalcone intermediates in the presence of hydroxylamine hydrochloride. The structures of the isoxazoline derivatives were confirmed by spectral analysis. The compounds were screened for their *in vitro* antibacterial activity using gram-positive bacteria and gram-negative bacteria. Compounds were also screened for their antifungal activity. Several derivatives of isoxazoline produced good to moderate activities against number of bacteria and fungus.

Keywords: isoxazoline, chalcone, antibacterial activity, heterocyclic synthesis

INTRODUCTION:

Compounds incorporating heterocyclic ring systems continue to attract considerable interest due to the wide range of biological activities they posses. Amongst them five membered heterocyclic compounds occupy a unique place in the realm of natural and synthetic organic chemistry. Five membered heterocycles like isoxazoline have found wide application as pharmaceutical and agrochemical agents. In recent years, attention has increasingly been given to the synthesis of isoxazoline derivatives as a source of new antibacterial agents. The synthesis of novel isoxazoline derivatives remain a main focus of medicinal research. Isoxazoline derivatives have been reported to possess antifungal [1], antibacterial [2], anticonvulsant [3], anti-inflammatory [4], anti-viral [5], analgesic [6], antitumor [7], chemotherapy [8] activity. Penicillin derivatives containing isoxazole ring were found to be antibacterial agent [9]. Isoxazoline derivatives also showed good potency in animal models of thrombosis [10]. In addition, isoxazoline derivatives have played a crucial role in the theoretical development of heterocyclic chemistry and are also used extensively in organic synthesis [11-15]. Encouraged by

the diverse biological activities of compounds; in this work isoxazoline derivatives were synthesized via cyclization of substituted chalcone intermediates in the presence of hydroxylamine hydrochloride, with an objective to develop potent anti-microbial agents.

MATERIALS AND METHODS:

Melting points were determined by Thieles tube method (Table 1) and were uncorrected. ^{1}H NMR spectra were recorded on Avance II 400 (Make; Bruker, France) NMR Spectrometer. FT-IR spectra were recorded on MB 3000 (Make; ABB Bomem, Canada) spectrometer.

Synthesis of substituted isoxazoline derivatives:

Step-1. Procedure for synthesis of substituted chalcone derivative:

A solution of sodium hydroxide (40%) in water and rectified spirit was placed in a flask provided with a mechanical stirrer. The flask was immersed in a bath of crushed ice. Substituted acetophenones (A1) (0.006M) was poured with constant stirring, benzofuran-2-carbaldehyde (B1) (0.006M) was added to the solution. The temperature of the mixture was kept at about 25^{0}C and was stirred vigorously until the mixture was thick enough to retard the stirring (4-6 hr). The stirrer was removed and the reaction mixture was kept at 8^{0}C overnight. The product (C1) was filtered with suction on a buchner funnel, washed with cold water until the washings were neutral to litmus and then with ice cold ethanol. The crude product was recrystallized from ethanol.

Step-2. Procedure for synthesis of substituted isoxazoline derivatives:

A mixture of substituted Chalcones (C1) and hydroxylamine hydrochloride in ethanol was taken in a round bottom flask. The reaction mixture was refluxed for 6 hrs on a water bath followed with addition of ice cold water at room temperature. The mixture was kept overnight at 8^{0}C. The precipitates were filtered, washed with distilled water and dried. The product was recrystallized with ethanol to get the final products (I1-I6).

Biological evaluation:

***In-vitro* Antimicrobial Screening:**

The *in vitro* antibacterial screenings of synthesized compounds were performed against the following standard bacterial strains: *Pseudomonas aeruginosa* (MTCC 424), *Escherichia coli* (MTCC 1573), *Bacillus subtilis* (MTCC 441) and *Staphylococcus aureus*

(MTCC 1430). For antifungal screening *Penicillium chrysogenum* (MTCC 161) and *Aspergillus niger* (MTCC 2546) were used.

Cylinder plate method:

A definite volume of the microbial suspension (inoculums) was poured into the sterilized nutrient agar media (cooled at 40ºC) and mixed thoroughly. About 20 ml of this suspension was poured aseptically in the petri plates and kept till the solidification. The surface of agar plates was pierced using a sterile cork borer. The prepared wells were filled with equal volume of a solution of synthesized compounds and standard drugs; separately. After a period of pre-incubation diffusion, the plates were incubated face up for a definite time under specified conditions. The zones of inhibition were measured as a parameter of antimicrobial properties of synthesized derivatives.

RESULTS AND DISCUSSION:

Novel isoxazoline derivative were synthesized by cyclization of substituted chalcone derivatives in the presence of hydroxylamine hydrochloride (Figure 1). Physiochemical properties of synthesized compounds were determined in terms of melting point & % yield (Table 1). Synthesized compounds were also characterized using FT-IR and ^{1}H-NMR. The IR spectrum of the synthesized compounds revealed presence of –OH (3402 cm^{-1} O-H str. of alcohol) and C=N (aromatic) stretching at 1515-1655 cm^{-1}, 735 cm^{-1} (C-Cl str.), 1348 cm^{-1} (C-O-N str. in ring), 851; 680 cm^{-1} (aromatic C-H def.) and 640 (C- Br str.), etc. In ^{1}H-NMR spectra δ value of various synthesized compounds was found in the range of 1.29-1.68 for methyl proton and 6.14-7.87 for benzyl proton (Table 2). The synthesized derivatives showed the presence of aromatic ring which was also evident in the ^{1}H NMR spectra. Antifungal & antibacterial activities were also performed as *in-vitro* antimicrobial screening against fungal strains & bacterial strain respectively (Table 3 & 4). According to preliminary antibacterial screening by paper disc method some compounds were found to have comparable antibacterial activity against *S. aureus*, *B. subtilis* and *E. coli* compared to Norfloxacin as a standard drug and for antifugal screening some compounds were found to active against *A. niger* and *P. chrysogenum* using Fluconazole as a standard drug. The antimicrobial screening revealed that the compound I6 and compound I4 exhibited potent antibacterial & antifungal activity respectively as compared to other derivatives. Compounds (I6) was found to exhibits potent *in-vitro* antibacterial activity against *Pseudomonas aeruginosa* and *Bacill subtilis* while compound (I4) was found to exhibit potent *in-vitro* anti-fungal activity against *Aspergillus niger* and *Penicillium chrysogenum.* (Table 3, Table 4).

Table 1. Physical constants of synthesized isoxazoline derivatives

Compound Code	Melting range (^{0}C)	% Yield	R_f-value (Mean±S.D.)
I1	111-114	70	0.77±0.01
I2	121-125	65	0.72±0.02
I4	117-119	80	0.61±0.06
I4	131-134	58	0.74±0.03
I5	124-128	87	0.81±0.04
I6	115-119	77	0.84±0.05

Table 2. Spectral analysis of the synthesized isoxazoline derivatives

Comp. code	IR spectra (cm^{-1})	^{1}H NMR Spectra (δ) in ppm
I1	1682 (C=N str. in ring), 1612,1599,1587,1487, 1445, 1462 (aromatic C=C str.), 1423 (C-N str.), 1348 (C-O-N str. in ring), 851,680 (aromatic C-H def.), 640 (C- Br str.).	7.61-8.51 (r, 4H, aromatic ring), 7.08-7.36 (s, 4H, aromatic ring), 4.5 (m, 1H, CH), 3.39-3.52 (s, 2H, CH_2 in ring).
I2	1682 (C=N str. in ring), 1593, 1568, 1491, 1437, 1408 (aromatic C=C str.), 1348 (C-O-N str. in ring), 851,680 (aromatic C-H def.), 735 (C- Cl str.).	7.61-8.51 (r, 4H, aromatic ring), 7.13-7.20 (s, 4H, aromatic ring), 4.5 (m, 1H, CH), 3.39-3.52 (s, 2H, CH_2 in ring).
I3	1640 (C=N str. in ring), 1595, 1585, 1539, 1495 (aromatic C=C str.), 1365 (C-O-N str. in ring), 1092 (N-C str. in dimethylamino), 833,684 (aromatic C-H def.).	6.80-7.20 (r, 4H, aromatic ring), 6.52-7.01 (s, 4H, aromatic ring), 5.0 (r, 1H), 2.85 (s, 6H, CH_3).
I4	3209 (O-H str.), 1670 (C=N str. in ring), 1612, 1585, 1490, 1469, 1448 (aromatic C=C str.), 1338 (C-O-N str. in ring), 1317 (aromatic C-OH str.), 831,684 (aromatic C-H def.).	6.76-7.20 (r, 4H, aromatic ring), 7.16-7.22 (s, 4H, aromatic ring), 5.66 (r, 1H, OH).
	2918 (aliphatic C-H str.), 1655 (C=N str. in ring), 1587, 1566, 1510, 1481, 1466, 1450	7.62-8.42 (r, 4H, aromatic ring), 6.77-7.10

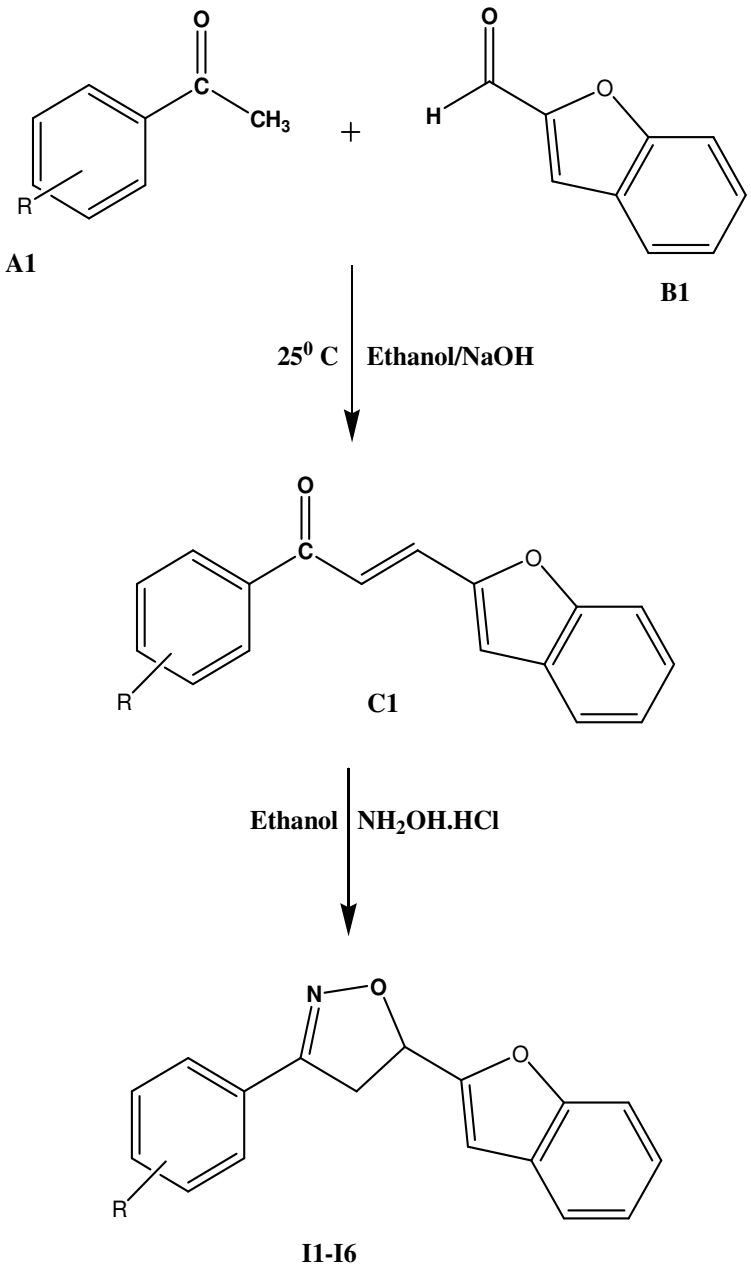
I5	(aromatic C=C str), 1340 (C-O-N str. in ring), 1252 (aromatic C-O str. in OCH_3), 820,695 (aromatic C-H def.).	(s, 4H, aromatic ring), 3.75 (s, 3H, CH_3).
I6	3000 (aliphatic C-H str. in CH_3), 1680 (C=N str. in ring), 1650, 1614, 1593, 1514, 1481 (aromatic C=C str.), 1348 (C-O-N str. in ring), 835,700 (aromatic C-H def.).	7.62-8.62 (r, 4H, aromatic ring), 6.99 -7.07 (s, 4H, aromaticring), 2.35 (s, 3H,CH_3).

Table 3. Antifungal Activity

Compounds	**Zone of Inhibition (mm)**	
	Penicillium chrysogenum (MTCC 161)	*Aspergillus niger* (MTCC2546)
I1	13	09
I2	12	15
I3	16	14
I4	18	17
I5	12	13
I6	11	16
Fluconazole	20	18

Table: 4. Antibacterial Activity

	Gram negative bacteria		**Gram positive bacteria**	
	Escherichia coli (MTCC 1573)	*Pseudomonas aeruginosa* (MTCC 424)	*Staplococc aureus* (MTCC 1430)	*Bacill subtilis* (MTCC 441)
Compounds	**Zone of Inhibition (mm)**			
I1	12	12	13	12
I2	17	10	10	16
I3	17	13	09	15
I4	14	15	12	14
I5	11	14	08	11
I6	17	19	18	18
Norfloxacin	21	19	21	23



Products ↓	R
I1	Br
I2	Cl
I3	N $(CH_3)_2$
I4	OH
I5	OCH_3
I6	CH_3

Fig. 1: Scheme for the synthesis of substituted isoxazoline derivatives

CONCLUSION:

Present research work involves synthesis of novel Isoxazoline derivative to explore their antimicrobial activity. Compound I6 exhibited highest antibacterial activity and compound I4 exhibited potent antifungal activity against bacterial and fungal strains

respectively. Hence, it is concluded that there is ample scope for further study in developing these as good lead compounds for the treatment of bacterial strain as well as fungal strain.

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