ANTI-BACTERIAL AND IN VITRO ANTI-DIABETIC POTENTIAL OF NOVEL ISOXAZOLE DERIVATIVES.

ABSTRACT

Aim: To synthesize novel Isoxazole derivatives,characterize them and subject for screening anti-bacterial action and in vitro anti-diabetic activity.

Methodology: Chalcones were prepared by the reaction of aromatic aldehydes with aromatic ketones in aqueous alcoholic alkaline medium. Then these were made to react with hydroxylamine hydrochloride and sodium acetate to prepare title compounds. The prepared isoxazole compounds were subjected to invitro anti-diabetic screening by yeast and enzymatic method. All compounds were screened for antibacterial action by disc diffusion method.

Result: The structure of the synthesized compounds were confirmed by IR, NMR spectral datas and screened for anti-bacterial, and anti-diabetic activities. Most effective antibacterial one possesed chlorine in the Phenyl ring attached at 5-C of isoxazole and have NH_2 substitution in phenyl ring attached at 3-C of isoxazole. Compounds with significant invitro anti-diabetic action by studying the glucose uptake by yeast cell method are with Br/NO_2 substituted forR_2/R_3 in phenyl ring when R'2 is OH/NH_2.

Conclusion: Presence of halogenated aromatic ring at 5-C and amine substituted phenyl ring at 3-Cof Isoxazole exhibited moderate anti-bacterial activity. In the anti-diabetic study halogenated or nitrated phenyl ring at 5-C and hydroxyl/amine substituted phenyl ring at 3-C of isoxazole exhibited anti-diabetic action.

Key words: Isoxazole, Chalcones, aromatic aldehyde, aromatic ketones, antibacterial, *in vitro* anti-diabetic\

INTRODUCTION

Isoxazole being an azole with an oxygen atom next to the nitrogen, exhibits broad spectrum of biological activity and also forms a part of various biodynamic agents. Substituted isoxazoles are

also considered to be important synthons due to their versatility towards chemical transformations to useful synthetic intermediates. A lot of modifications have been done during the last few years on isoxazole nucleus. A survey of literature revealed that substituted isoxazole possess different types of potent biological activities[1-3].

Many Isoxazole derivatives are stated to have good antimicrobial activity[4-6]. Many studies indicate isoxazole derivatives improved diabetic condition[7-8]. In this light hereby prepared many Isoxazole derivatives which screened for anti-bacterial activity and *in vitro* antidiabetic activity.

METHODOLOGY

Preparation of chalcones

0.01mol of benzaldehyde was taken and added 0.01 mol acetophenone in 10 ml 95% ethanol in a flask. 3.5 ml 6M NaOH solution was added to the reaction mixture stirred well for 10 minutes. Cooled in ice bath until crystal formation. 2 ml ice cold water added to it followed by 2 ml ice cold ethanol. Allow to air dry. Recrystallise from ethanol.

Cyclisation step

The formed unstable chalcones were further cyclised with 0.015 mol of hydroxylamine hydrochloride and sodium acetate 0.015mol in 25 ml ethanol was refluxed for 6 hrs. The mixture was concentrated and poured in to ice. The precipitate obtained was filtered washed and recrystallised from ethanol.

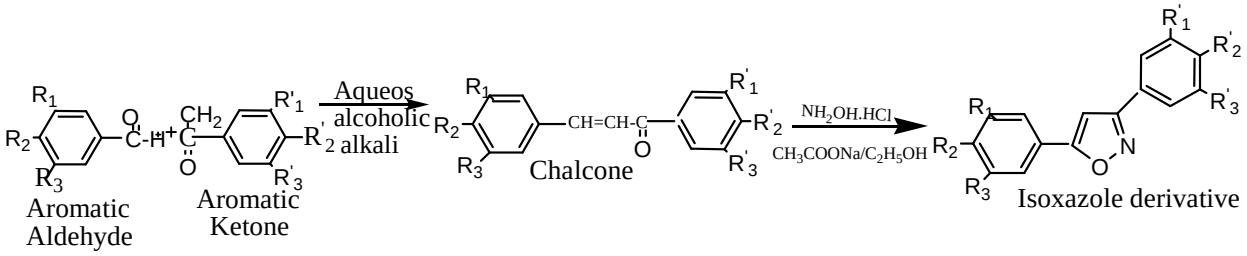

Figure:1-scheme of synthesis of the isoxazole derivatives

In vitro anti-diabetic screening:

a) Glucose uptake in Yeast cells[9]:

Yeast cells were prepared by, commercial baker's yeast and was washed by repeated centrifugation (3,000×g; 5 min) in distilled water until the supernatant fluids were clear and a10% (v/v) suspension was prepared in distilled water. Various concentrations of extracts (1–5 mg) were added to 1 mL of glucose solution (5, 10 and 25 mM) and incubated together for 10 min at 37 °C. Reaction was started by adding 100 micro liter of yeast suspension, vortex and further incubated at 37 °C for 60 min. After 60 min, the tubes were centrifuged (2,500 × g, 5 min) and glucose was estimated in the supernatant. The percentage increase in glucose uptake by yeast cells was calculated using the following formula-

Increase in glucose uptake (%) = Abs sample– Abs control* 100 / Abs sample

Where, Abs control is the absorbance of the control reaction (containing all reagents except the test sample), and Abs sample is the absorbance of the test sample. All the experiments were carried out in triplicates.

b) Alpha-amylase inhibitory activity[10]:

The activity of α-amylase was measured using the starch-iodine method. Briefly, 20 µ l of α-amylase solution (0.030 mg/ml) was mixed with 1.3 ml of Tris-HCl buffer (0.01 M containing 0.006 M NaCl, pH 6.8) and 80 micro liter of the aqueous extract. After incubation at 37ºC for 20 min, 100 micro liter of the starch solution (0.1%) was added, and the mixture re-incubated for 20 min, after which 2 ml of 0.01% acidic iodine solution was added, and the absorbance measured at 565 nm. The percentage inhibition was calculated by comparing to the control which did not have the extract. Inhibition of enzyme activity was calculated as (%)

(A-C) × 100/ (B-C) , where, A = absorbance of the sample, B = absorbance of blank (no extract), and C = absorbance of control (no starch).

Determination of antibacterial activity

The antibacterial activity of synthetic products was assessed against three bacteria species: *Bacillus subtilis* NCIM 2063 ,*Staphylococcus aureus* NCIM 2079 and *Escherichia coli* NCIM 2931. The respective cultures were prepared in nutrient broth media after incubation at 37 °C for 24 hrs. .The antibacterial activity was determined by agar disc diffusion test.

Agar disc diffusion test[11]

Using an aseptic technique, place a sterile swab into the broth culture of a specific organism and then gently remove the excess liquid by gently pressing or rotating the swab against the inside of the tube. Using the swab, streak the Nutrient agar plate to form a bacterial lawn. To obtain uniform growth, streak the plate with the swab in one direction, rotate the plate 90° and streak the plate again in that direction. Repeat this rotation 3 times. Allow the plate to dry for approximately 5 minutes.

Use an Antibiotic Disc Dispenser to dispense discs onto the plate and administer the respective samples onto the labelled discs. Using a flame-sterilized forceps, gently press each disc to the agar to ensure that the disc is attached to the agar. Plates should be incubated overnight at an incubation temperature of 37 °C.

RESULTS AND DISCUSSION:

Table:1- IR and NMR Spectral details of synthesized compounds

Sample.ID	**IR peaks(cm^{-1})**	**NMR peaks (ppm)**
L1	1011(N-O-stretch),1500(C-O-stretch),1243 (N-O out of pane bend)	11.663(alcoholic proton)8-421 (2H),-CH_2-5 membered ring,8.330,(1H)-isomer of isoxazole, 8.229, 8.2210,8.05,8.04-(4H),of Aro-H.
L2	1104(N-O- stretch),1511(C-O stretch), 1245(N-O out of pane bend)	8.404-(2H),-CH2(5-membered ring).8.357-(1H)isomer of isoxazole,8.363,8.366,8.369-(4H,Ar-H)
L3	1105(N-O stretch),1533(C-O stretch), 1350(N-O out of pane bend)	11.646-alcoholic proton., 8.424-(2H,CH2-5 membered ring),
L4	1018(N-O stretch),1500(C-O stretch),1201(N-O out of pane bend)	8.337-(2H),CH2(5-membered ring)7.469-(4H) of Ar-H
L5	1018 (N-O stretch),1500(C-O stretch), 1201(N-O out of pane bend)	7.470 (4H)of Ar-H
L6	1016(N-O stretch),1530(C-O stretch), 1219(N-O out of plane bend)	11.075 – alcoholic proton peak.8.141-(2H)CH2-5-membered ring,7.959,7.955,7.875 (4H,of Ar-H)
L7	1009(N-O stretch),1550(C-O stretch), 1217(N-O out of plane bend)	11.844 alcoholic proton,8.308-(2H)CH2-5 membered ring.7.872,7.850-(4H,Ar-H)
L8	1171(N-O stretch),1517(C-O-stretch), 1245(N-O out of plane bend)	10.966-alcoholic proton,8.306-(2H,CH2-5 membered ring),8.067,7.545,7.539 (4H of Ar-H)

L9	1041(N-O stretch),1491(C-O stretch), 1295(N-O out of plane bend)	11.186-alcoholic proton peak.7.526-(2H,CH2 5-membered ring)
L10	1090(N-O stretch),1490(C-O stretch), 1219(N-O out of plane bend)	8.176-(2H,CH2-5membered ring),8.170-OH isomer of isoxazole.8.157,8.152,8.149-Ar-H
L11	1012(N-O stretch),1490(C-O stretch),1219(C-N stretch),1100(N-O out of plane)	8.175-2H (CH2-of 5-membered ring.)8.157-OH isomer of isoxazole.8.153,7.995-(4H of Ar-H)
L12	1012 (N-O stretch),1218(C-O stretch),1489(C-N stretch),1100(N-O out of plane bend)	8.175- (2H (CH2-of 5-membered ring.)8.157-OH isomer of isoxazole.8.154,7.996-(4H of Ar-H))
L13	1116(N-O stretch),1240(C-O stretch), 1550(C-O carbonyl),1100(N-O out of plane)	8.231- (2H (CH2-of 5-membered ring.)8.227-OH isomer of isoxazole.8.223,7.=8.204-(4H of Ar-H))
L14	1012(N-O stretch),1219(C-N stretch), 1490(C-N stretch)	8.177- (2H (CH2-of 5-membered ring.)8.174-OH isomer of isoxazole.8.153,7.996-(4H of Ar-H))
L15	1040(N-O stretch),1530(C-O-stretch), 1178(C-N stretch)	8.766- (2H (CH2-of 5-membered ring.)8.341-OH isomer of isoxazole.8.321,8.272,8.269-(4H of Ar-H))
L16	1008(N-O stretch),1223(C-N stretch), 1603(C-O stretch)	8.172- (2H (CH2-of 5-membered ring.)8.150-OH isomer of isoxazole.8.004,7.965-(4H of Ar-H))
L17	1016(N-O stretch),1218(C-N stretch), 1527(C-O stretch)	8.793- (2H (CH2-of 5-membered ring.)8.788-OH isomer of isoxazole.8.784,8.362-(4H of Ar-H))
L18	1047(N-O stretch),1260(C-N stretch), 1490(C-O stretch),1179(N-O out of plane)	8.173- (2H (CH2-of 5-membered ring.)8.151-OH isomer of isoxazole.7.990,7.951,7.936-(4H of Ar-H))
L19	1041(N-O stretch),1585(C-O stretch),1183(N-O out of plane)	7.746- (2H (CH2-of 5-membered ring.)7.725-OH isomer of isoxazole.7.707,7.674,7.635-(4H of Ar-H))
L20	1046(N-O stretch),1212(C-N stretch), 1507(C-O stretch),1153(N-O out of plane)	8.749- (2H (CH2-of 5-membered ring.)8.114-OH isomer of isoxazole.8.089,8.070,8.037-(4H of Ar-H))
L21	1041(N-O stretch),1264(C-N stretch), 1519(C-O stretch)	8.294- (2H (CH2-of 5-membered ring.)8.272-OH isomer of isoxazole.8.201,8.177-(4H of Ar-H))
L22	1000(N-O stretch),1507(C-O stretch), 1238(C-N stretch)	8.052- (2H (CH2-of 5-membered ring.) 6.914--(4H of Ar-H))
L23	1039(N-O stretch),1129(C-Nstretch), 1492(C-O stretch)	7.503- (2H (CH2-of 5-membered ring.)7.500-OH isomer of isoxazole.7.497,7.494,7.490-(4H of Ar-

		H))
L24	1051(N-O stretch),1213(C-N stretch), 1507(C-O stretch)	8.320- (2H (CH2-of 5-membered ring.)7.831-OH isomer of isoxazole.7.810,7.691,7.686-(4H of Ar-H)
L25	1060-(N-O stretch),1270(C-N stretch), 1550(C-O stretch)	8.732- (2H (CH2-of 5-membered ring.)8.066-OH isomer of isoxazole.8.032,7.946,7.926-(4H of Ar-H))

Table:2- Elemental analysis result of synthesized compounds

CALCULATED				ANALYTICAL		
Sample	**N%**	**C%**	**H%**	**N%**	**C%**	**H%**
L1	4.43	56.99	3.19	4.65	66.76	3.86
L2	9.92	63.83	3.57	10.59	49.60	3.81
L3	5.16	66.31	3.71	7.67	77.11	8.42
L4	5.27	76.48	5.21	8.45	51.10	3.58
L5	4.43	56.99	3.19	4.65	66.50	3.67
L6	9.92	63.83	3.57	10.64	49.87	2.79
L7	9.92	63.83	3.57	5.20	67.00	4.02
L8	4.71	68.68	5.09	4.65	66.98	4.30
L9	4.71	68.68	3.85	5.61	67.46	5.80
L10	4.58	58.85	2.96	3.80	54.30	3.60
L11	4.46	61.17	3.85	4.62	66.74	4.90
L12	9.99	68.56	4.32	10.62	49.81	3.02
L13	5.19	71.25	4.48	8.48	50,91	3.77
L14	5.62	81.90	6.06	5.19	67.85	3.75
L15	4.46	61.17	3.85	4.60	67.39	5.47
L16	9.99	68.56	4.32	10.64	68.50	6.02
L17	9.99	68.56	4.32	10.60	49.68	4.27
L18	4.07	59.32	4.10	4.10	69.71	5.16
L19	9.03	65.80	4.55	5.64	78.69	6.90
L20	4.32	61.83	4.51	3.24	59.18	4.44
L21	8.89	57.16	3.52	6.31	46.17	2.60
L22	4.89	55.71	6.08	5.96	50.98	7.98
L23	10.35	66.55	4.10	11.03	70.76	6.03
L24	10.35	66.55	4.10	10.69	68.89	6.38
L25	11.19	76.78	5.64	10.67	68.74	6.42

None of the tested compounds are superior to Ciprofloxacin which was the standard. Most effective one possesed chlorine in the Phenyl ring attached at 5-C of isoxazole and have NH_2 substitution in phenyl ring attached at 3-C of isoxazole against Gram +ve *Bacillus subtilis*, *staphylococcus aureous* and Gram –ve *E.coli*. A few compounds substituted with nitro/halogen on Phenyl ring at 5-C of isoxazole and ethoxyl group on phenyl ring at 3-C of isoxazole observed to be active only against *E.coli* . Compounds with significant in vitro anti-diabetic action by studying the glucose uptake by yeast cell method are with Br/NO_2 substituted forR_2/R_3 in phenyl ring when R'2 is OH/NH_2.

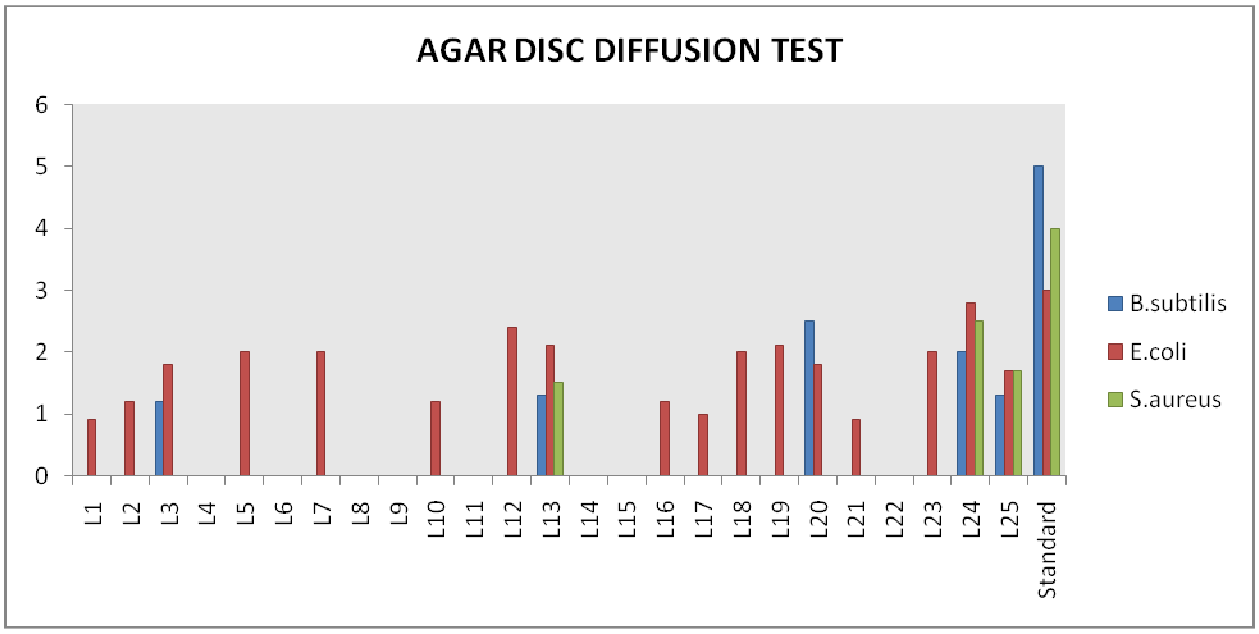

Figure 2:Anti-bacterial activityof Isoxazole compounds by disc diffusion method

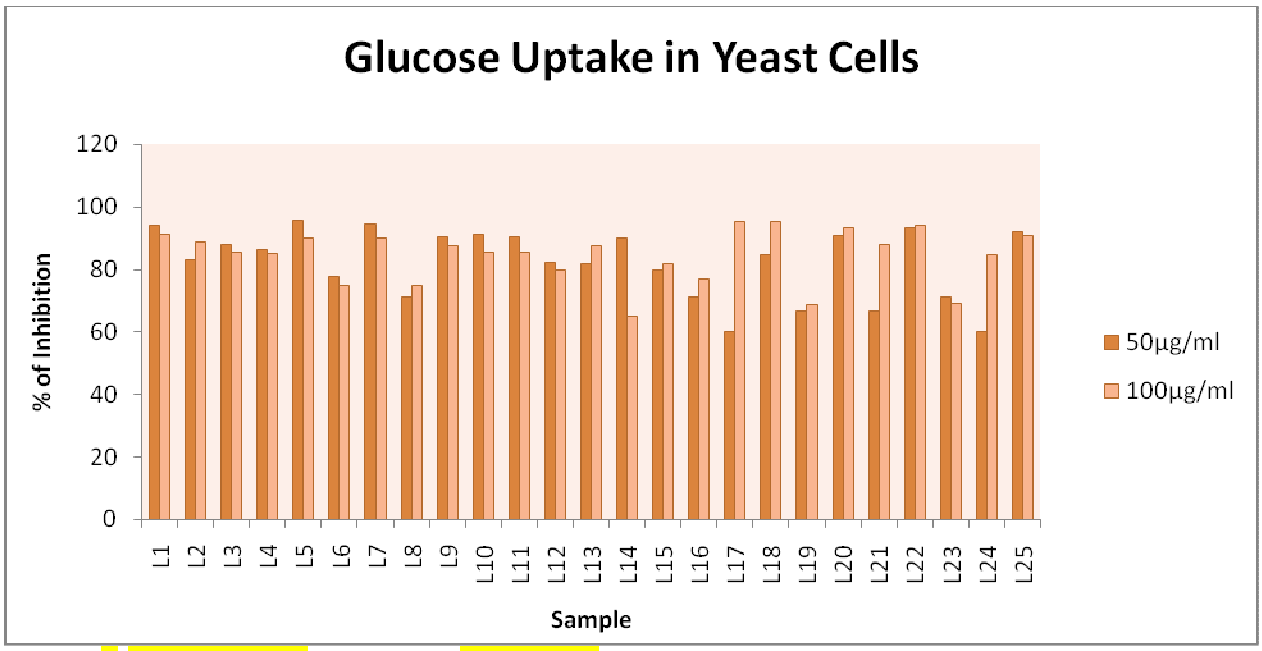

Figure 3:Anti-diabetic activity of Isoxazoles by studying glucose uptake in living cells

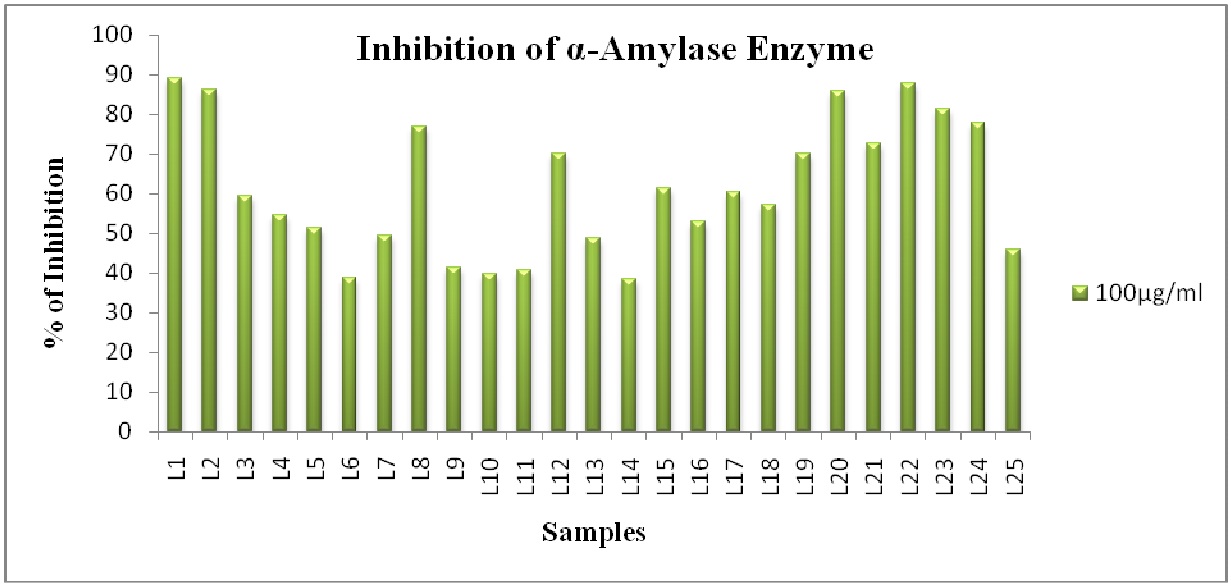

Figure 4: anti-diabetic activity of Isoxazoles by inhibition of α amylase enzyme

Conclusion: Presence of halogenated aromatic ring at 5-C and amine substituted phenyl ring at 3-Cof Isoxazole exhibited moderate anti-bacterial activity. In the anti-diabetic study halogenated

or nitrated phenyl ring at 5-C and hydroxyl/amine substituted phenyl ring at 3-C of isoxazole exhibited anti-diabetic action.

Disclaimer: - . " Abstract of the work was preliminary reported on the Drug Discovery Conference, August 27-28 2015, Riga, Latvia."

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