



Amylase & Lipase Inhibitory Effects and Antioxidant Effects of Novel Oxazolines



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Abstract

Most of the studies reveal the inference of oxidative stress in diabetes pathogenesis by the alteration in enzymatic systems, lipid peroxidation, impaired Glutathione metabolism and decreased Vitamin C levels. By seeing the significance of Amylase & Lipase inhibitory effects and antioxidant effects in reducing the burden of Diabetes, herewith aimed to synthesize oxazolines and to screen for Amylase & Lipase inhibitory effects and antioxidant effects. Twenty five Oxazoline derivatives are prepared, characterized and screened for the anti oxidant, lipase and amylase inhibitory activity. Out of 25 synthesized compounds seven compounds shows significant amylase inhibitory activity, twelve compounds shows significant antioxidant and lipase inhibitory activity. Dimethoxyl group, halogen or nitro group at para position and dimethyl amino substitution enhance amylase inhibitory effect. Most of the compounds exhibited lipase inhibitory effects have significant anti-oxidant action.

Keywords : Oxazolines; Pancreatic lipase; Amylase

Introduction

Oxazoline play a major role in medicinal chemistry. These are five membered heterocycle with one double bond and oxygen and nitrogen as hetero atoms. There are many natural and synthetic molecules which contain oxazoline nucleus having biological significance. The nitrogen present in Oxazoline is basic in nature. The synthesis, structures and biological activities of oxazoline derivatives have long been focused of research interest of organic chemists in the field of medicine due to the potential biological activities exhibited by them [1]. Alpha-amylases hydrolyze complex polysaccharides to produce oligosaccharides and disaccharides which are then hydrolyzed by α -glucosidase to monosaccharides which are then absorbed through the small intestines into the hepatic portal vein. Inhibitors of α -amylase and/or α -glucosidase such as acarbose, miglitol and voglibose [2] are known to lower postprandial hyperglycemia (PPHG) in type 2 diabetic patients by delaying digestion and subsequent absorption of carbohydrates from the gut. Newer approaches for the treatment of obesity have involved inhibition of dietary triglyceride absorption via inhibition of pancreatic lipase.

Prevention of digestion of digestive lipids could be an effective strategy for preventing systemic absorption of lipids. Any imbalance between the free radicals and antioxidants leads to produce a condition known as "oxidative stress" that results in the development of pathological condition among which one

is diabetes. Most of the studies reveal the inference of oxidative stress in diabetes pathogenesis by the alteration in enzymatic systems, lipid peroxidation, impaired Glutathione metabolism and decreased Vitamin C levels. Lipids, proteins, DNA damage, Glutathione, catalane and superoxide dismutase are various biomarkers of oxidative stress in diabetes mellitus. Reactive oxygen (ROS) and nitrogen (RNS) species include superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hypochlorite (ClO^-), hydroxyl radical (OH^\bullet), nitric oxide (NO), and peroxynitrite ($ONOO^-$) [3]. In physiological conditions, mitochondria are the major site of intracellular ROS production, due to electron leakage along the respiratory chain; nevertheless, they can also arise from plasma membrane systems, endoplasmic reticulum, lysosomes, peroxisomes and cytosolic enzymes.

At low concentrations, ROS/RNS exert a multitude of biological effects, including immune-mediated defense against pathogenic microorganisms and intracellular signaling; conversely, high levels of these extremely reactive species can damage DNA, lipids, and proteins, thus leading to tissue injury and cell death. Endogenous antioxidant compounds are urate, glutathione, ubiquinone, and thioredoxin; furthermore, some proteins (ferritin, transferrin, lactoferrin, caeruloplasmin) act as antioxidants, as they bind and sequester transition metals that may start oxidative reactions. Antioxidant enzymes are

superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase, glutathione S-transferase, catalase, thioredoxin reductase, peroxiredoxins (Prx), and NAD(P) H:ubiquinone oxidoreductase (NQO1). Obesity may develop oxidative stress and it is the major cause of metabolic syndrome. Here we are making an attempt to find a lead molecule with desired effects like anti-amylase, anti-lypase and anti-oxidant effects.

Materials and Method

Preparation of sample

Proposed compounds prepared by making reaction between substituted aromatic aldehydes and dimethylamine in presence of Pyridinium Hydrobromide PerBromide. The resultant mixture is washed with sodiumthiosulphate and successively with sodium chloride. The resultant product recrystallised from ethanol. The structure of obtained compounds characterized and screened for anti-amylase, anti-lipase and anti-oxidant effects.

Evaluation of Lipase Inhibitory Activity

Pancreatic lipase inhibitory properties have been extensively examined for the determination of the potential effect of these oxazoline derivatives as antiobesity agents. Acinar cells of pancreas secrete an enzyme pancreatic lipase and it releases fatty acids from the triglyceride skeleton at the C-1 and C-3 position. These fatty acids are incorporated into bile acid-phospholipid micelles and further absorbed at the small intestine, and finally enter to the peripheral circulation as chylomicrons. Interference with fat hydrolysis leads to decreased utilization of ingested lipids; hence, lipase inhibition reduces fat absorption. Lipase activity was assayed by turbidimetric method using olive oil-ethanol suspension. This suspension was prepared by mixing 1 ml of olive oil to 100 ml of ethanol and shaken vigorously. 1mL of this olive oil-ethanol suspension was added to 9 mL of 0.05 M Tris-HCl buffer, pH 8.0 containing 0.025M sodium deoxycholate. This emulsion was used as substrate. Reaction mixture containing enzyme and inhibitor (in requisite amount) was incubated at room temperature for 10 min. Reaction was started by addition of 1 ml of substrate. Incubated for 10 min at 37°C. The decrease in turbidity was measured at 660 nm. Inhibitors present in the reaction prevented the decrease of turbidity of the mixture. Suitable 'control' tubes were run parallel [4].

Evaluation of anti oxidant activity

Human body is rich in free radical. The ability of oxazoline derivatives to scavenge hydrogen peroxide was determined according to the method of Ruch. A solution of 40 mM hydrogen peroxide was prepared in phosphate buffer (pH 7.4). 100 µg / ml prepared compound in distilled water were added to a hydrogen peroxide solution (0.6 mL, 40mM). Absorbance of hydrogen peroxide at 230 nm was determined after 10 minutes against a blank solution containing the phosphate buffer without hydrogen peroxide [5].

$$\% \text{ Scavenged } [H_2O_2] = [(A_c - A_s) / A_c] \times 100$$

Here AC and AS are the absorbance of control and absorbance of test/standard respectively.

In -Vitro Alpha-amylase inhibitory assay

Alpha amylase and alpha glucosidases are responsible for the breakdown of oligosaccharides/disaccharides to monosaccharide. So inhibitor of this enzyme delay carbohydrate metabolism which results in marked decrease in glucose absorption. To 500 µl of (100µg/ml) test compounds, 500 µl of starch in phosphate buffer (pH 6.9) containing 6.7mM of sodium chloride was added. The reaction was initiated by adding 500 µl porcine pancreatic amylase and incubated at 37°C. To the above mixture add 1ml of DNSA (1g of DNSA, 30g of sodium potassium tartarate and 20 mL of 2N sodium hydroxide) was added and made up to a final volume of 100 mL with distilled water and kept it in boiling water bath for 5 minutes and cooled to room temperature. The reaction mixture diluted with 10 ml of distilled water and absorbance was read at 540 nm. Blank tubes were prepared by replacing the enzyme solution with 500 µL in distilled water. Control, representing 100% enzyme activity was prepared in a similar manner, without sample. Maltose is used as standard [6].

$$\text{Inhibition (\%)} = \frac{(\text{Abs of Control} - \text{Abs of test}) \times 100}{\text{Abs of Control}}$$

Statistical Evaluation

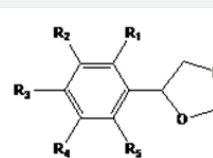


Figure 1: General Structure for scheme I Products.

All results were expressed as mean ± standard deviation (n=3). Significance of difference from the control was determined by dunnet test (one way ANOVA), Twenty five Oxazoline derivatives are prepared, characterized and among the tested compounds LJ₁₁, LJ₁₂, LJ₁₅, LJ₁₆, LJ₁₇, LJ₂₂, LJ₂₃ found to have maximum Amylase inhibitory effects (Figure 1 & 2). LJ₂, LJ₄, LJ₈, LJ₁₀, LJ₁₁, LJ₁₂, LJ₁₅, LJ₁₆, LJ₁₇, LJ₂₂, LJ₂₃, LJ₂₄ found to have maximum lipase inhibitory activity (Figure 3). LJ₁, LJ₇, LJ₈, LJ₁₀, LJ₁₁, LJ₁₂, LJ₁₄, LJ₁₅, LJ₁₆, LJ₁₈, LJ₂₀, LJ₂₃ (Figure 4) found to have maximum anti oxidant activity. Amylase inhibitory effects, that may be attributed to the presence of hydroxyl/methoxyl/halogen/ nitro/dimethylamino functional groups at 3rd/4th position in phenyl ring. The compounds with more lipase inhibitory effects didn't show any specific pattern of presence of certain functional groups. In the case of antioxidant activity screening phenyl ring attached with halogenated/methylated/ hydroxylated showed promising activities.

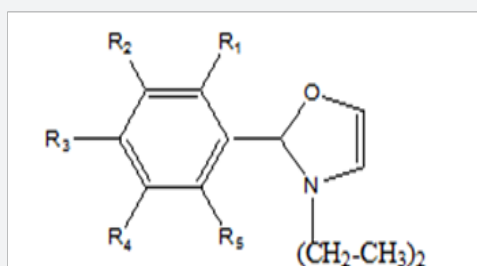


Figure 2: General Structure for scheme II Products.

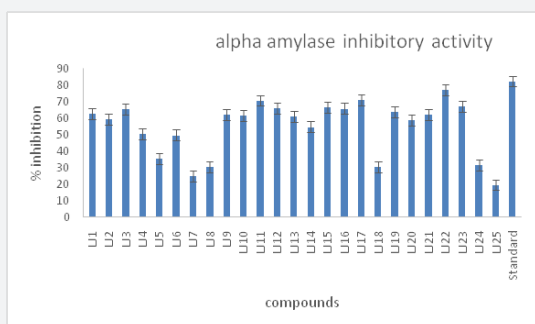


Figure 3: Effect of novel oxazoline derivatives on alpha amylase inhibitory activity (In-vitro).

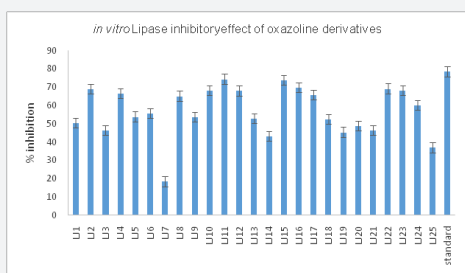


Figure 4: Effect of novel oxazoline derivatives on lipase inhibitory activity (In-vitro).

Results and Discussion

Twenty five Oxazoline derivatives are prepared, characterized and among the tested compounds LJ₁₁, LJ₁₂, LJ₁₅, LJ₁₆, LJ₁₇, LJ₂₂, LJ₂₃ (Table 1 & 2) found to have maximum Amylase inhibitory effects (Figure 1 & 2). LJ₂, LJ₄, LJ₈, LJ₁₀, LJ₁₁, LJ₁₂, LJ₁₅, LJ₁₆, LJ₁₇, LJ₂₂, LJ₂₃, LJ₂₄ found to have maximum lipase inhibitory activity (Figure 5). LJ₁, LJ₇, LJ₈, LJ₁₀, LJ₁₁, LJ₁₂, LJ₁₄, LJ₁₅, LJ₁₆, LJ₁₈, LJ₂₀, LJ₂₃ (Table 3) (Figure 4) found to have maximum anti oxidant activity. Amylase inhibitory effects, that may be attributed to the presence of hydroxyl/methoxyl/halogen/ nitro/dimethylamino functional groups group at 3rd/4th position in phenyl ring. The compounds with more lipase inhibitory effects didn't show any specific pattern of presence of certain functional groups. In the case of antioxidant activity screening phenyl ring attached with halogenated/ methylated/ hydroxylated showed promising activities (Table 4).

Table 1: Structure of synthesized novel oxazoline derivatives.

Sample	R ₁	R ₂	R ₃	R ₄	R ₅
LJ1	H	H	Cl	H	H
LJ2	NO ₂	H	H	H	H
LJ3	H	H	NO ₂	H	H
LJ4	H	H	H	Br	H
LJ5	H	H	H	Cl	H
LJ6	CH ₃	H	H	H	H
LJ7	H	Br	H	H	H
LJ8	H	CH ₃	H	H	H
LJ9	H	H	N-(CH ₃) ₂	H	H
LJ10	H	H	OH	OH	H
LJ11	H	H	OCH ₃	OCH ₃	H
LJ12	H	OCH ₃	H	OCH ₃	H
LJ13	H	OCH ₃	OCH ₃	OCH ₃	H
LJ14	H	Cl	H	H	H
LJ15	H	H	F	H	H
Sample	R ₁	R ₂	R ₃	R ₄	R ₅
LJ16	H	H	Cl	H	H
LJ17	H	Cl	H	H	H
LJ18	NO ₂	H	H	H	H
LJ19	H	H	F	H	H
LJ20	OCH ₃	H	OCH ₃	H	H
LJ21	H	OCH ₃	OCH ₃	OCH ₃	H
LJ22	H	H	N-(CH ₃) ₂	H	H
LJ23	H	H	NO ₂	H	H
LJ24	H	OCH ₃	OCH ₃	H	H
LJ25	Cl	H	H	H	H

Table 2: Effect of novel oxazoline derivatives on alpha amylase inhibitory activity (in-vitro). The values are expressed in average ± standard deviation. n=3**P<0.01; *P<0.05.

No	Compound	Percentage
1	LJ1	62.6±0.987
2	LJ2	59.5±0.587
3	LJ3	65.29±0.995
4	LJ4	50.53±0.893
5	LJ5	35.33±0.958
6	LJ6	49.68±0.897
7	LJ7	24.87±0.882
8	LJ8	30.6±0.645
9	LJ9	62.03±0.236
10	LJ10	61.72±0.539
11	LJ11	70.73±0.852*
12	LJ12	66.06±0.583
13	LJ13	60.99±0.891
14	LJ14	54.72±0.536

15	LJ15	66.59±0.784*
16	LJ16	65.79±0.793*
17	LJ17	71.13±0.992**
18	LJ18	30.62±0.149
19	LJ19	63.99±0.387
20	LJ20	58.62±0.789*
21	LJ21	62.05±0.879*
22	LJ22	77.16±0.877**
23	LJ23	67.34±0.576
24	LJ24	31.64±0.853
25	LJ25	19.55±0.558
26	Standard	82.39±0.897**

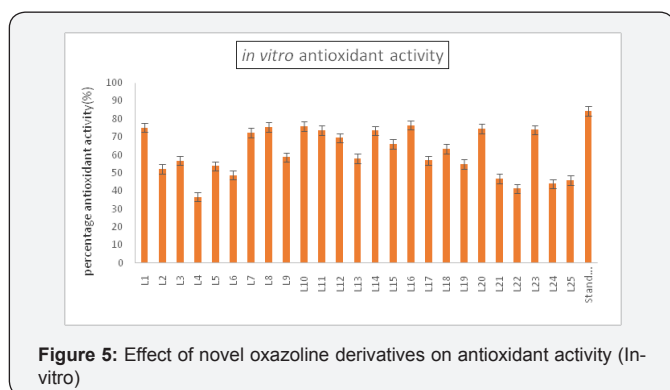


Figure 5: Effect of novel oxazoline derivatives on antioxidant activity (In-vitro)

Table 3: Effect of novel oxazoline derivatives on lipase inhibitory activity (in-vitro). The values are expressed in average ± standard deviation. n=3**P<0.01; *P<0.05.

No	Compound	Percentage Inhibition
1	LJ1	50.4±0.684
2	LJ2	69.09±0.754
3	LJ3	46.4±0.564
4	LJ4	66.59±0.875
5	LJ5	53.85±0.724
6	LJ6	55.65±0.975
7	LJ7	18.410±0.897
8	LJ8	65.17±0.578
9	LJ9	53.77±0.984
10	LJ10	68.24±0.856
11	LJ11	74.44±0.954**
12	LJ12	68.09±0.594
13	LJ13	52.92±0.589
14	LJ14	43.19±0.957
15	LJ15	73.8±0.879
16	LJ16	69.93±0.437*
17	LJ17	65.9±0.957
18	LJ18	52.53±0.785
19	LJ19	45.38±0.847

20	LJ20	48.89±0.578
21	LJ21	46.4±0.986
22	LJ22	69.170.587*
23	LJ23	68.17v0.657*
24	LJ24	60.23±0.285
25	LJ25	36.93±0.587
27	standard	78.5±0.901**

Table 4: Effect of novel oxazoline derivatives on antioxidant activity (in-vitro) The values are expressed in average ± standard deviation. n=3**P<0.01; *P<0.05.

Compound	Percentage
LJ1	72.7±0.525*
LJ2	69±0.610
LJ3	57.1±0.824
LJ4	73±0.5177*
LJ5	74±0.501*
LJ6	57±0.801
LJ7	62.9±0.714
LJ8	66.4±0.6459
LJ9	55.6±0.8547
LJ10	79.3±0.587**
LJ11	69±0.487
LJ12	74.6±0.7352**
LJ13	52.7±0.9101
LJ14	53±0.899
LJ15	71.5±0.5487*
LJ16	55.3±0.8597
LJ17	58.5±0.7992
LJ18	63.9±0.694
LJ19	55.6±0.8549
LJ20	56.6±0.8361
LJ21	52.2±0.921
LJ22	52.6±0.921
LJ23	49±0.9785
LJ24	62±0.958
LJ25	48.9±0.548
Standard	84.9±0.875**

Conclusion

There are many natural and synthetic molecules which contain oxazoline nucleus having biological significance. The synthesis, structures and biological activities of oxazoline derivatives have long been focused of research interest of organic chemists in the field of medicine. Due to the potential biological activities exhibited by them. There is some sort of association observed between Anti-oxidant and Lipase inhibitory effect. Among the substituent's Dimethoxyl, Dimethylamino and halogens enhanced Amylase inhibitory effect.

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Author Biography

Dr.Lincy Joseph has completed her PhD in Pharmaceutical Sciences from Vinayaka Mission University .She is currently working in Pushpagiri college of Pharmacy, Kerala-India as Professor in Medicinal Chemistry. She has published more than 150 papers in reputed journals .She is a member of many professional bodies of Pharmaceutical Sciences. She has presented research papers abroad. She has authored textbooks too. She got awarded as eminent teacher and Best teacher Pharmacist recently.

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