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## **Design, synthesis and evaluation of phenyl oxazolone derivatives as cardioprotective drugs**

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Received: 10-10-2014 / Revised: 01-11-2014 / Accepted: 12-11-2014

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### **ABSTRACT**

In the present study oxazolone derivatives were prepared with substitution of amine propanol side chain at position 4 of 2-phenyl-5-oxazolones. The synthesized compounds were characterized on basis of spectral studies and explored for cardioprotective effect. Substituted oxazolone derivatives have been prepared by condensation of hippuric acid with p-amino benzaldehyde in presence of ethanol, acetic anhydride and sodium acetate. The prepared intermediate fused with epichlorhydrin moiety to synthesize 4-[2-(oxiran-2-yl methylamino)benzylidene]-2-phenyloxazol-5-(4H)-one. Then five different amino derivatives (VIA-VIE) were synthesized. Furthermore, the synthesized compounds were evaluated for cardioprotective activity. The structures of the synthesized compounds were assigned on the basis of UV, IR, and <sup>1</sup>H NMR data. The synthesized compounds were at dose level of 50 mg/kg body weight screened for cardioprotective activity and compared to standard ramipril at dose level of 1 mg/kg body weight. From the above studies it could be concluded that condensed product oxazolone and amino propanol showed significant (P<0.05) cardioprotective effect.

Key words: Heterocycles, Oxazolone, Cardioprotective, Spectroscopy, Ramipril,



### **INTRODUCTION**

Cyclic organic compounds in which the rings include at least one atom of an element different from the rest are known as heterocyclic compounds. Nitrogen, oxygen, sulfur are the most common heteroatoms. A considerable part of research carried out in chemistry is devoted to the study of heterocyclic compounds because these compounds occur widely in nature and plays very vital role in the synthesis of various biologically active agents [1]. Heterocyclic compounds containing nitrogen, oxygen belonging to five/ six membered heterocyclic compounds has occupied enormous significance in the field of medicinal chemistry. Nitrogen containing heterocyclic molecules constitute the largest portion of received in the chemical entities, which are part of many natural products, fine chemicals and biologically active pharmaceuticals and are vital for enhancing

the quality of life [2-3]. In the past few decades, the synthesis of new heterocyclic compounds has been a subject of great interest because of their wide applicability such as antianginal [4], antiarrhythmic [5], antihyperlipidemic [6], antiplatelets [7], analgesic [8], anti-inflammatory [9], antidiabetic, and antiobesity [10-12] and now much studied myocardial infarction.

Among the heterocycles, oxazolone belong to a class of five member heterocyclic compounds. Which are important intermediates in the synthesis of several small molecules including amino acids, peptides, heterocyclic precursors for biosensors coupling and photosensitive composition device for proteins and a wide range of pharmaceutical properties. Oxazolone is a chemical allergen used for immunological experiments particularly for experiments on delayed type hypersensitivity [13]. It was also found that certain oxazolone derivatives

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were reported to possess anticonvulsant [14], cardiotonic [15], vasorelaxant [16], anticancer [19, 20] and antimicrobial activities [21]. In view of this, it was considerable interest to synthesize the substituted phenyl oxazolone compounds with a hope to obtained potent biologically active compound.

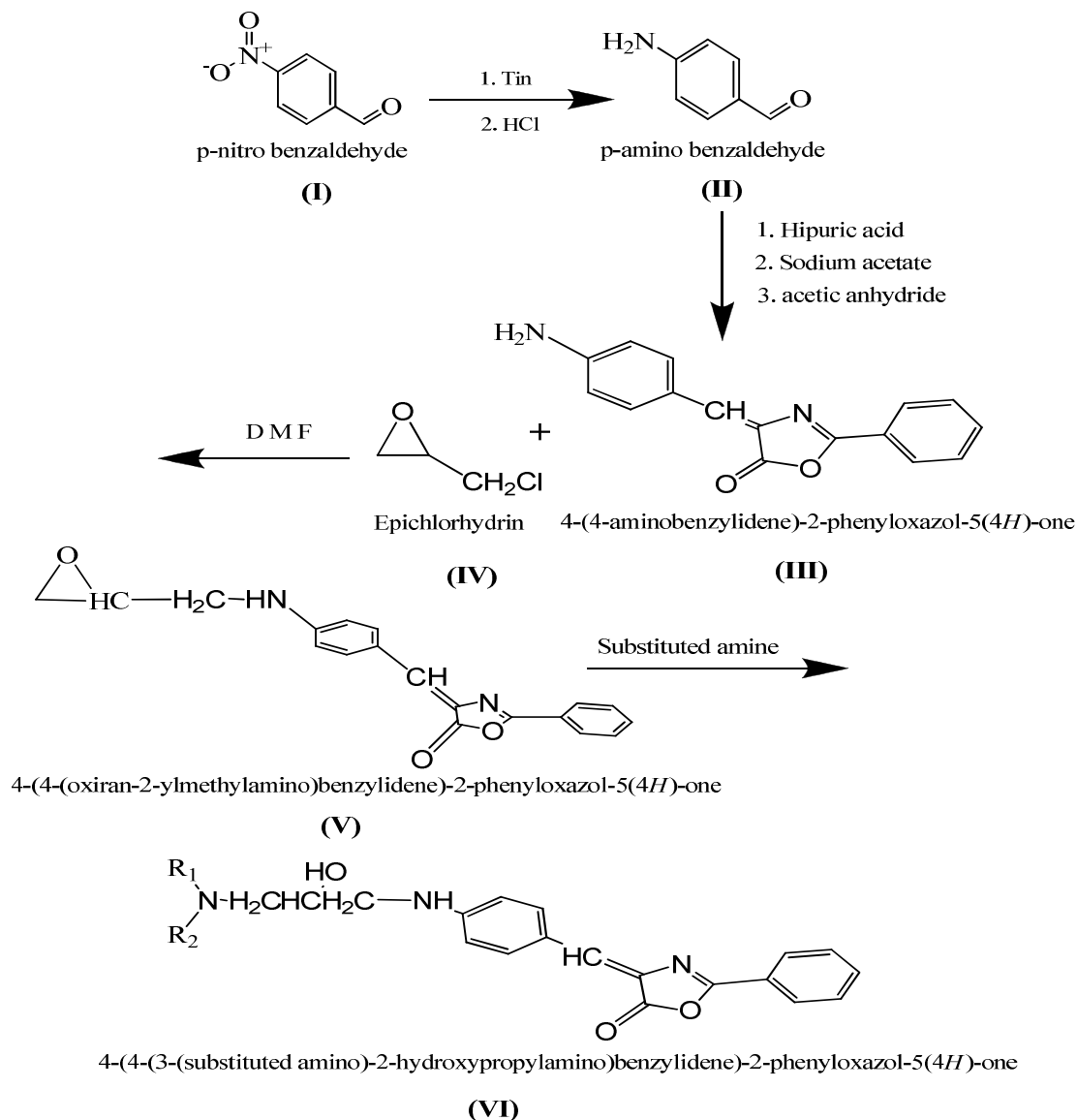
The experimental work was described under two parts:

Part 1: Synthesis and characterization of oxazolone derivatives and it required reagents and solvents (Table No.1), equipments, schematic representation and general procedure for synthesis of compounds.

Part 2: Pharmacological studies:

## MATERIALS AND METHODS

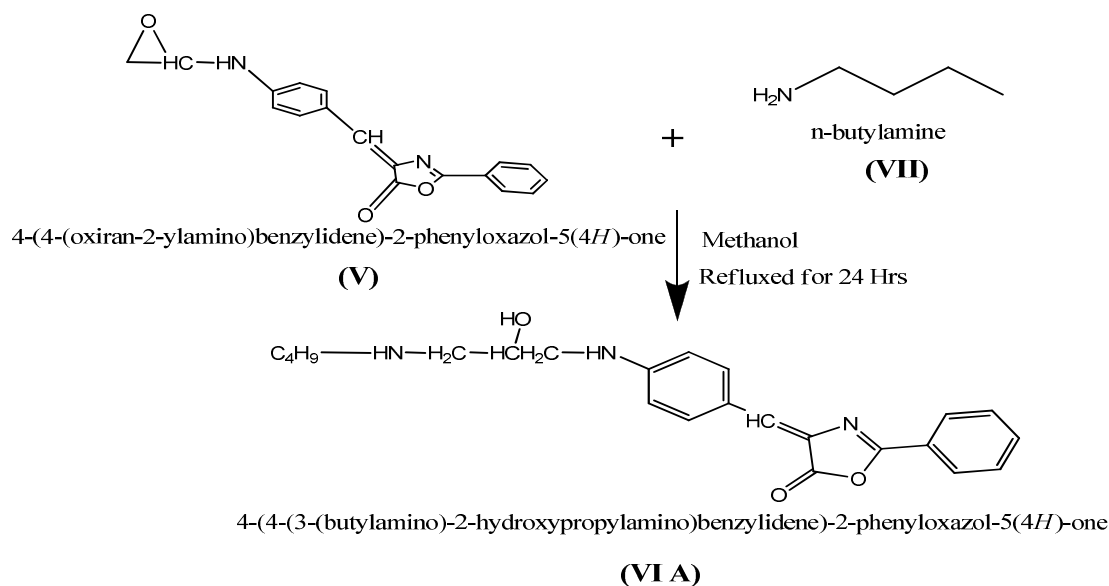
### General methodology



	R <sub>1</sub>	R <sub>2</sub>
1. (VI A)	H	CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub>
2. (VI B)	H	CH <sub>3</sub>
3. (VI C)	C <sub>2</sub> H <sub>5</sub>	C <sub>2</sub> H <sub>5</sub>
4. (VI D)	CH <sub>3</sub>	CH <sub>3</sub>
5. (VI E)	H	C <sub>2</sub> H <sub>5</sub>

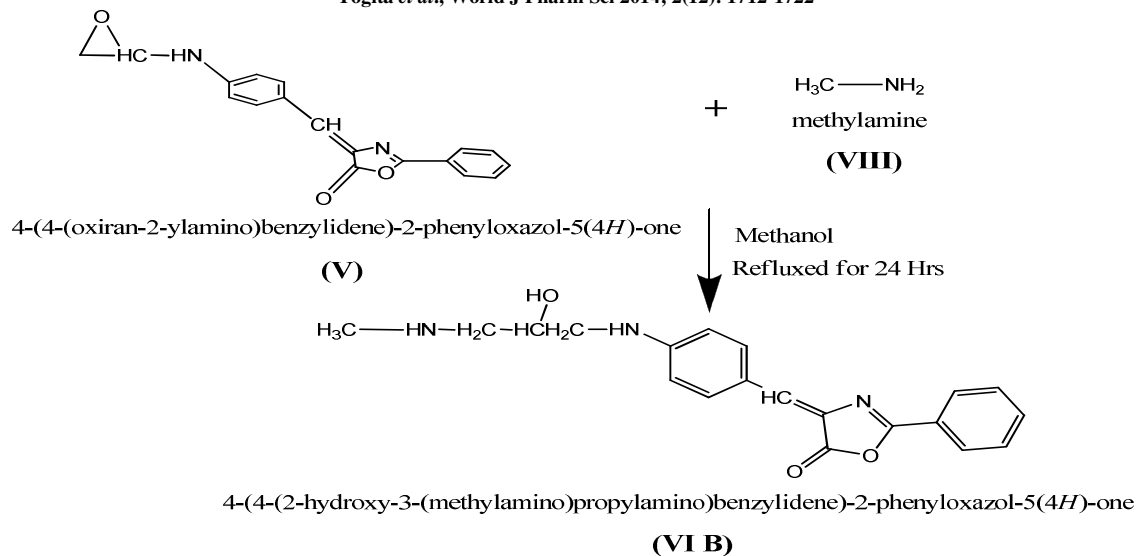
1. Synthesis of 4-(4-(3-(butylamino)-2-hydroxypropylamino)benzylidene)-2-phenyloxazol-5(4H)-one (VI A) from 4-(4-(oxiran-2-ylmethylamino)benzylidene)-2-phenyloxazol-5(4H)-one (V) and n-butylamine (VII): A solution of lead moiety 4-[4-(oxiran-2-ylmethylamino)benzylidene]-2-phenyloxazol-5-(4H)-

one (V) (0.013 mol) and n-butylamine (VII) (0.013 mol) in 40 ml of methanol was refluxed for 24 h. The product obtained was filtered, vacuum dried and recrystallized using ethanol. Brown crystal was obtained. The yield of final product was 80%.



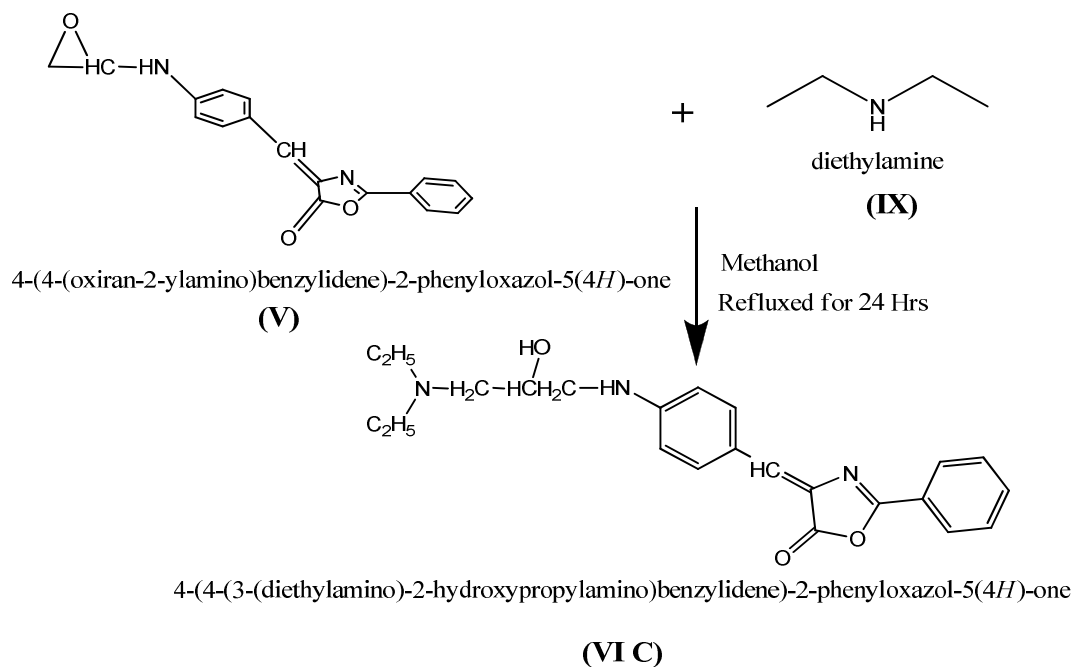
2. Synthesis of 4-(4-(3-(methylamino)-2-hydroxypropylamino)benzylidene)-2-phenyloxazol-5(4H)-one (VI B) from 4-(4-(oxiran-2-ylmethylamino)benzylidene)-2-phenyloxazol-5(4H)-one (V) and methylamine (VIII): A solution of lead moiety 4-[4-(oxiran-2-yl

methylamino)benzylidene]-2-phenyloxazol-5-(4H)-one (V) (0.013 mol) and methylamine (VIII) (0.013 mol) in 40 ml of methanol was refluxed for 24 h. The product obtained was filtered, vacuum dried and recrystallized by methanol. 83 % yield was obtained and colour of product was yellow.



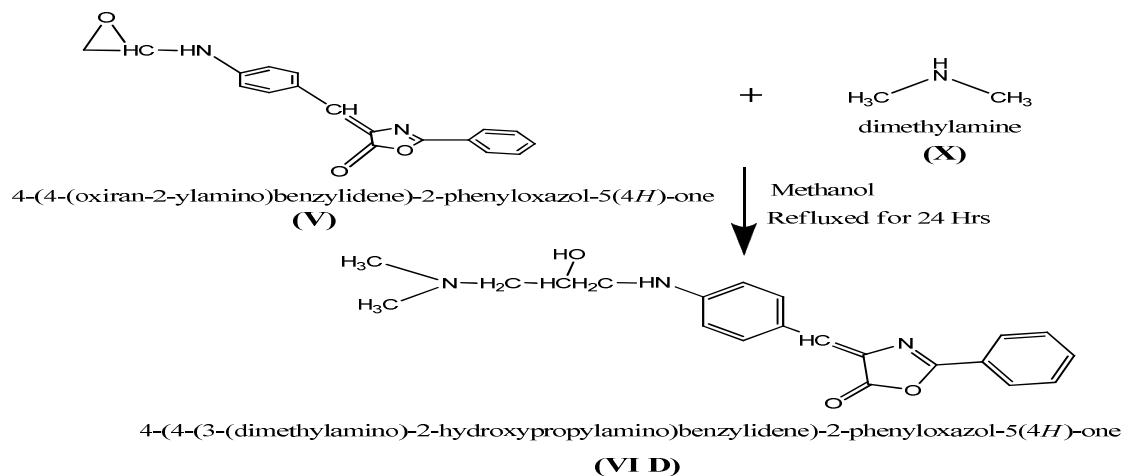
**3. Synthesis of 4-(4-(3-(diethylamino)-2-hydroxypropylamino)benzylidene)-2-phenyloxazol-5(4H)-one (VI C) from 4-(4-(oxiran-2-ylmethylamino)benzylidene)-2-phenyloxazol-5(4H)-one (V) and diethylamine (IX):** A solution of lead moiety 4-[4-(oxiran-2-yl

methylamino)benzylidene]-2-phenyloxazol-5-(4H)-one (VI) (0.013 mol) and dimethylamine (IX) (0.013 mol) in 40 ml of methanol was refluxed for 24 h. The product obtained was filtered, vacuum dried and recrystallized by ethanol. Deep brown colour product was obtained.



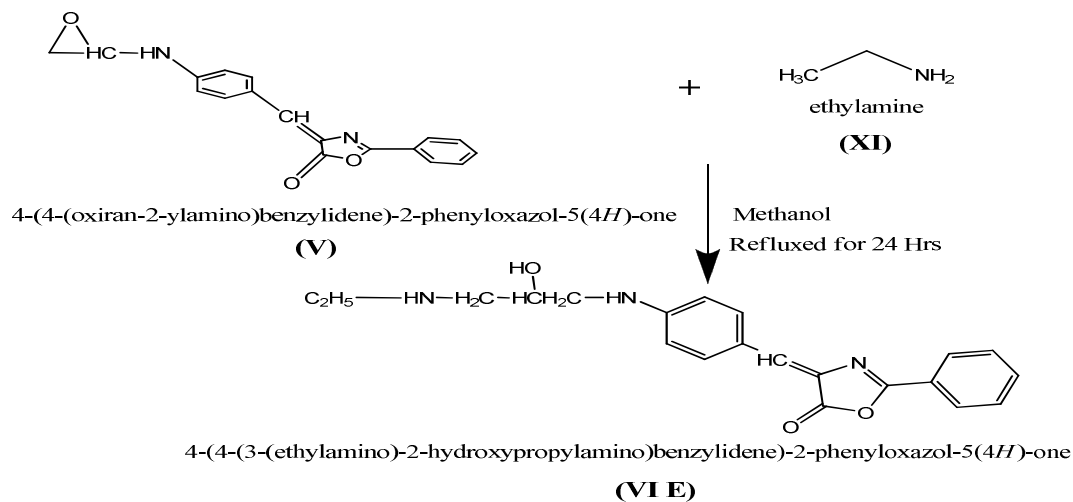
**4. Synthesis of 4-(4-(3-(dimethylamino)-2-hydroxypropylamino)benzylidene)-2-phenyloxazol-5(4H)-one (VI D) from 4-(4-(oxiran-2-ylmethylamino)benzylidene)-2-phenyloxazol-5(4H)-one (V) and dimethylamine (X):** A solution of lead moiety 4-[4-(oxiran-2-yl

methylamino)benzylidene]-2-phenyloxazol-5-(4H)-one (V) (0.013 mol) and dimethylamine (X) (0.013 mol) in 40 ml of methanol was refluxed for 24 h. The product obtained was filtered, vacuum dried and recrystallized by methanol. Reddish brown colour product was obtained.



**5. Synthesis of 4-(4-(3-(ethylamino)-2-hydroxypropylamino)benzylidene)-2-phenyloxazol-5(4H)-one (VI E) from 4-(4-(oxiran-2-ylmethylamino)benzylidene)-2-phenyloxazol-5(4H)-one (V) and dimethylamine (XI):** A solution of lead moiety 4-[4-(oxiran-2-yl

methylamino)benzylidene]-2-phenyloxazol-5(4H)-one (V) (0.013 mol) and ethylamine (XI) (0.013 mol) in 40 ml of methanol was refluxed for 24 h. The product obtained was filtered, vacuum dried and recrystallized by methanol. Yellow colour crystal was obtained.



## PHARMACOLOGICAL STUDY

**Animals:** The study was carried out on healthy Wistar albino rats weighing between 200-300 g of either sex. All the animals were maintained on rat feed and water *ad libitum*. They were housed in departmental animal house and were exposed to 12h cycle of light and dark. The rat were selected and divided by randomization into 8 groups (n=5). The experimental protocol used in the present study was approved by Institutional Animal Ethical Committee (IAEC/273/CPCSEA/10/II/2132/07).

## (VI E)

**Drugs and dose:** All the standard and test drugs were dissolved in 0.9% w/v normal saline. Test compounds were given at a dose of 50 mg/kg, p.o [22].

**Isolated rat heart preparation:** Heparin (500 I.U, i.p.) was administered to the rates 20 min before their scarifies by cervical dislocation. Hearts from heparinized rats were rapidly excised and immediately mounted on Langendorff's apparatus. The heart was perfused retrogradely at a coronary flow rate of 7-9 mL min<sup>-1</sup> at a constant pressure of 80 mmHg, with Kreb's-Henseleit (KH) buffer (NaCl 118 mM; KCl 4.7 mM; CaCl<sub>2</sub> 2.5 mM;

MgSO<sub>4</sub>.7H<sub>2</sub>O 1.2 mM; NaHCO<sub>3</sub> 25 mM; KH<sub>2</sub>PO<sub>4</sub> 1.2 mM; C<sub>6</sub>H<sub>12</sub>O<sub>6</sub> 11m M), pH 7.4, maintained at 37°C, bubbled with 95% O<sub>2</sub> and 5% CO<sub>2</sub>. Global ischaemia was produced for 30 min by blocking the inflow of Kreb's-Henseleit solution. It was followed by reperfusion for 120 min. Coronary effluent was collected before ischemia, immediately, 5 min and 30 min after reperfusion for estimation of Lactate Dehydrogenase (LDH) and Creatine Kinase-MB (CK-MB) [23].

**Assessment of infarct size:** Heart was removed from Langendorff's apparatus. Both the auricles and the root of aorta was excised and ventricles were kept overnight at a temperature of - 4°C. Frozen ventricles were sliced into uniform sections of about 1-2 mm thickness. The slices were incubated in 1% w/v triphenyl tetrazolium chloride (TTC) at 37°C in 0.2 M Tris buffer (pH 7.4) for 30 min. Dehydrogenase enzyme and cofactor NADH present in the viable myocardium react with tetrazolium salts to form a formazon pigment which is intensely colored. The enzyme and the cofactor are lost from the infarcted cardiac cells. Therefore, infarcted portion remains unstained

while the normal myocardium was stained brick red with TTC. Infarct size was measured by the volume method [24].

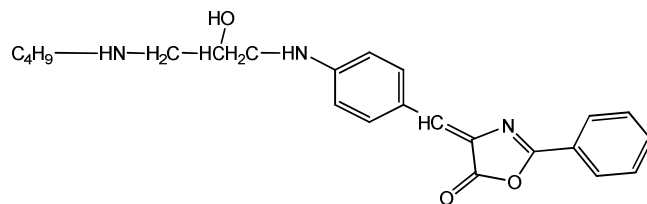
**Assessment of myocardial injury:** The myocardial infarct size was measured using the triphenyl tetrazolium chloride (TTC) staining method while the level of LDH and CK-MB (Siemens medical solution diagnostics Ltd., Baroda, India) in the coronary effluent was estimated using commercially available kits. Values of LDH and CK-MB were expressed in international units (IU) per liter [25].

## RESULTS AND DISCUSSION

**Characterization of synthesized compounds:** According to the scheme, different oxazolone derivatives were prepared. The characterization data of final compounds (VI A-VI E) are given in table (Table No. 2) respectively. The synthesized compounds are confirmed on the basis of spectral analysis.

### Spectral analysis of synthesized compounds

#### 1. VI A (C<sub>23</sub>H<sub>27</sub>N<sub>3</sub>O<sub>3</sub>):



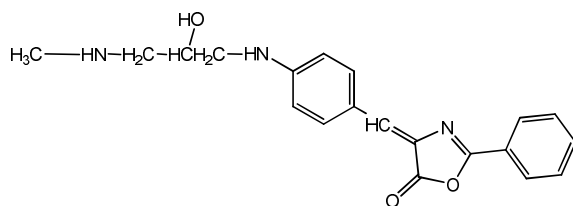
4-(4-(3-(butylamino)-2-hydroxypropylamino)benzylidene)-2-phenyloxazol-5(4H)-one

**U V Spectral analysis :** 261 nm.

**IR Spectral analysis :** 3401.99(>N-H amine), 3336.67(C-H aromatic), 2727.12(C-H aliphatic), 3002.33(CH<sub>2</sub>), 2665.65(CH<sub>3</sub>), 1788.23(C=O), 1255.11(>N-amines).

**NMR Spectral analysis :** 8.058 δ (s, 1H, =CH-C<sub>6</sub>H<sub>5</sub>); 7.716 -7.956 δ (m, 9H, Ar-H); 3.995 δ (s, 1H, CH-OH); 3.688-3.664 δ (s, 2H, -CH<sub>2</sub>-NHCH<sub>3</sub>); 3.949-3.972 δ (s, 2H, CH<sub>2</sub>-NH); 3.663 & 3.686 δ (s, 1H, -N<); 3.363-3.607 δ (s, 9H, C<sub>4</sub>H<sub>9</sub>-N-); 4.719 δ (s, 1H, OH)

#### VI B (C<sub>20</sub>H<sub>21</sub>N<sub>3</sub>O<sub>3</sub>):



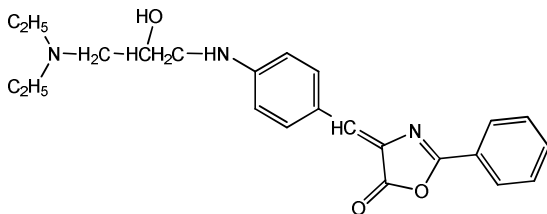
4-(4-(2-hydroxy-3-(methylamino)propylamino)benzylidene)-2-phenyloxazol-5(4H)-one

**UV Spectral analysis** : 287 nm.

**IR Spectral analysis:** 3485.96(>N-H amine), 3323.34(C-H aromatic), 2721.65(C-H aliphatic), 2941.34(CH<sub>2</sub>), 2610.32(CH<sub>3</sub>), 1764.60(C=O), 1249.62(>N-amines).

**NMR Spectral analysis:** 8.076 δ (s, 1H, =CH-C<sub>6</sub>H<sub>5</sub>); 7.704 -7.886 δ (m, 9H, Ar-H); 3.977 δ (s, 1H, CH-OH); 3.682-3.664 δ (s, 2H, -CH<sub>2</sub>-NHCH<sub>3</sub>); 3.955-3.977 δ (s, 2H, CH<sub>2</sub>-NH); 3.638-3.850 δ (s, 1H, -N<); 2.658 δ (s, 3H, CH<sub>3</sub>-N-); 4.722 δ (s, 1H, OH)

**VI C (C<sub>23</sub>H<sub>27</sub>N<sub>3</sub>O<sub>3</sub>) :**



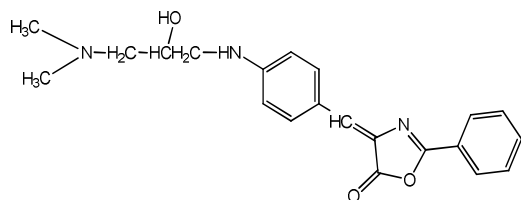
4-(4-(3-(diethylamino)-2-hydroxypropylamino)benzylidene)-2-phenyloxazol-5(4H)-one

**UV Spectral analysis** : 303 nm.

**IR Spectral analysis** : 3178.22(>N-H amine), 3076.16(C-H aromatic), 2812.43(C-H aliphatic), 2967.12(CH<sub>2</sub>), 2623.13(CH<sub>3</sub>), 1786.98(C=O), 1250.50(>N-amines).

**NMR Spectral analysis** : 8.082 δ (s, 1H, =CH-C<sub>6</sub>H<sub>5</sub>); 7.662-7.999 δ (m, 9H, Ar-H); 3.960 δ (s, 1H, CH-OH); 3.689-3.664 δ (s, 2H, -CH<sub>2</sub>-NHCH<sub>3</sub>); 3.933-3.960 δ (s, 2H, CH<sub>2</sub>-NH); 3.638 & 3.689 δ (s, 1H, -N<); 1.118-1.109 δ (s, 10H, C<sub>4</sub>H<sub>10</sub>-N-); 4.115 δ (s, 1H, OH)

**VI D (C<sub>21</sub>H<sub>23</sub>N<sub>3</sub>O<sub>3</sub>) :**



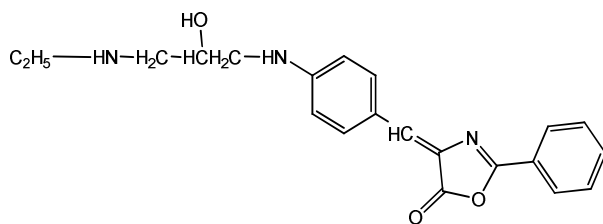
4-(4-(3-(dimethylamino)-2-hydroxypropylamino)benzylidene)-2-phenyloxazol-5(4H)-one

**UV Spectral analysis** : 334 nm

**IR Spectral analysis** : 3322.76(>N-H amine), 3276.50(C-H aromatic), 2886.45(C-H aliphatic), 3154.22(CH<sub>2</sub>), 2790.44(CH<sub>3</sub>), 1743.33(C=O), 1234.87(>N-amines).

**NMR Spectral analysis** : 8.106 δ (s, 1H, =CH-C<sub>6</sub>H<sub>5</sub>); 7.681 -7.881 δ (m, 9H, Ar-H); 3.950 δ (s, 1H, CH-OH); 3.689-3.654 δ (s, 2H, -CH<sub>2</sub>-NHCH<sub>3</sub>); 3.950-4.205 δ (s, 2H, CH<sub>2</sub>-NH); 3.638 & 3.708 δ (s, 1H, -N<); 2.758-2.739 δ (s, 6H, C<sub>2</sub>H<sub>6</sub>-N-); 4.708 δ (s, 1H, OH)

VI E (C<sub>21</sub>H<sub>23</sub>N<sub>3</sub>O<sub>3</sub>) :



4-(4-(3-(ethylamino)-2-hydroxypropylamino)benzylidene)-2-phenyloxazol-5(4H)-one

UV Spectral analysis : 299 nm

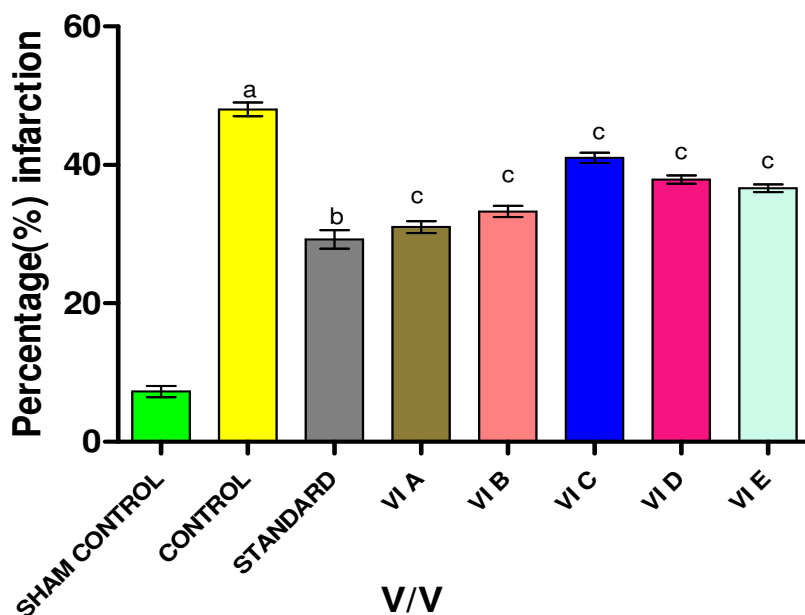
IR Spectral analysis : 3434.65(>N-H amine), 3345.98(C-H aromatic), 2643.11(C-H aliphatic), 2990.33(CH<sub>2</sub>), 2611.32(CH<sub>3</sub>), 1799.11(C=O), 1255.24(>N-amines).

NMR Spectral analysis : 8.112 δ (s, 1H, =CH-C<sub>6</sub>H<sub>5</sub>); 7.672 -7.989 δ (m, 9H, Ar-H); 3.994 δ (s, 1H, CH-OH); 3.912-3.753 δ (s, 2H, -CH<sub>2</sub>-NHCH<sub>3</sub>); 2.121-3.525 δ (s, 2H, CH<sub>2</sub>-NH); 3.618 & 3.607 δ (s, 1H, -N<); 1.157 δ (s, 2H, C<sub>2</sub>H<sub>5</sub>-N-); 4.385 δ (s, 1H, OH)

#### Pharmacological study of synthesized compounds

**Assessment of myocardial infarct size:** The various synthesized compounds were evaluated in ischemia and reperfusion induced increase in myocardial infarct size. As shown in figure no-1 the test comp VI A significantly attenuated

ischaemia and reperfusion induced increase in myocardial infarct size as compared to the control group. However, treatment with standard (Ramipril, 1mg/kg) was significantly more effective to reduce myocardial infarct size as compared to test compound VI A measured by macroscopic volume method.

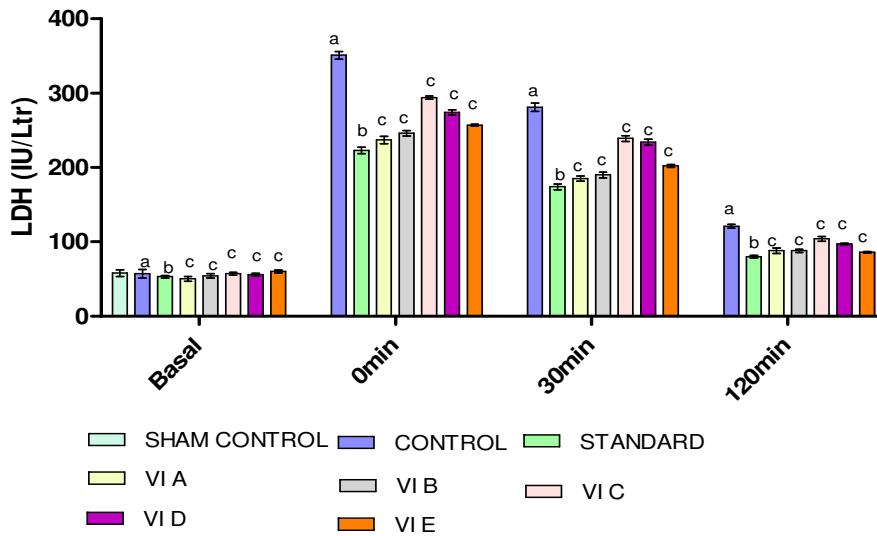


**Figure 1: Effect of Compound VI A on Myocardial Infarct Size.** Infarct size was measured by volume method. Values are expressed as mean ±SEM. a= P <0.05 vs. Sham control; b= P <0.05 vs. Control; c= P <0.05 vs. Standard. ANOVA followed by Tukey's multiple comparison test.



**Estimation of LDH release:** The various synthesized compounds were evaluated on ischemia and reperfusion induced increase in release of LDH in coronary effluent measured immediately (0min), 30 min and 120 min after reperfusion. As shown in figure no-2 the VI A significantly reduced ischaemia and reperfusion

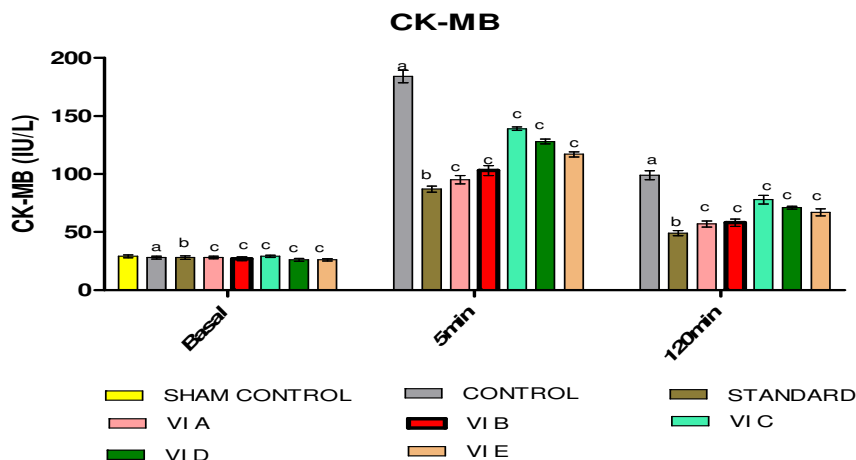
induced increases in release of LDH in coronary effluent measured immediately (0min), 30 min and 120 min after reperfusion. Moreover, treatment with standard (Ramipril, 1mg/kg) markedly reduced release of LDH in coronary effluent as compared to VI A measured immediately (0 min), 30 min and 120 min after reperfusion.



**Figure 2: Effect of Compound VI A on LDH release.** LDH was estimated in coronary effluent after stabilization (Basal), Immediately (Imm'Rep.) and 30 min. and 120 min. after reperfusion. Values are expressed as mean  $\pm$ SEM. a= P <0.05 vs. Sham control; b= P <0.05 vs. Control; c= P <0.05 vs. Standard. ANOVA followed by Tukey's multiple comparison test.

**Estimation of CK-MB release IU/L:** The various synthesized compounds were evaluated on ischemia and reperfusion induced increase in release of CK-MB in coronary effluent measured 5 min and 120 min after reperfusion. As shown in figure no-3 the synthesized compound VI A significantly reduced ischaemia and reperfusion

induced increases in release of CK-MB in coronary effluent measured 5 min and 120 min after reperfusion. Moreover, treatment with standard (Ramipril, 1 mg/kg) markedly reduced release of CK-MB in coronary effluent as compared to VI A measured 5min and 120 min after reperfusion.



**Figure 3: Effect of Compound VI A on CK-MB release.** CK-MB was estimated in coronary effluent after stabilization (Basal), 5 min. and 120 min. after reperfusion. Values are expressed as mean  $\pm$ SEM. a= P <0.05 vs. Sham control; b= P <0.05 vs. Control; c= P <0.05 vs. Standard. ANOVA followed by Tukey's multiple comparison test.

## CONCLUSION

Wide varieties of pharmaceutical activity are associated with heterocyclic compounds. Although there are many reports of heterocycles being used as cardioprotective agents but it requiring search of more novel heterocyclic compounds, which can be claimed as cardioprotective drugs. Since recent years have witnessed a steady increase in the incidence of cardiovascular disease, which, according to an estimate, are responsible for about 30% of total untimely death. Coronary heart diseases, reported to be fifth leading cause of death in the year 1990 by the World Health Organization, are estimated to top the list of cause of untimely death by the year 2020. Myocardial infarction occurs when a coronary artery has been blocked by thrombus. This may be fatal and is a common cause of death, usually as a result of mechanical failure of the ventricle or from dysrhythmia.

Huge number of drugs is used in cardiac problem as cardioprotective, but not even a single drug has reached the satisfactory result. So this becomes prime and important worldwide research to carry in search of the potent cardioprotective drug. In view of this there are reports where heterocycles especially oxazolones are possessing good cardioprotective action. On through literature survey, it was found that beta blockers are possessing amine propanol side chain necessary for their action. Therefore in present study, oxazolone

derivatives were prepared with substitution of amine propanol side chain at 4<sup>th</sup> position of 2-phenyl-5-oxazolone to see better effect in terms of reduction in myocardial infarct size, release of LDH and CK-MB level in coronary effluent.

Attempt were made to synthesized phenyl substituted oxazolones with aminopropanol side chain and evaluating their effect on ischemia/reperfusion induced myocardial injury by estimating the level of CK-MB and LDH in coronary fluid. Also change in the myocardial infarct size was assessed macroscopically by volume method. From the above studies compound VI A with substitution at C-4 by benzylidene and at C-4 by 3-(butylamino amino)-2-hydroxy propyl amino side chain showed maximum effect as compared to other in the series. Therefore, it could be concluded that substituted phenyl oxazolone with aminopropanol as a side chain possesses good cardioprotective effect. Moreover, some other biomarkers which are also present in the myocardium could be estimated with these derivatives, further the toxicity study, in vitro hydrolytic pattern, mechanism of action and clinical trials of these derivatives could be studied in future for introducing the new cardioprotective product in the market. Also some novel derivatives of oxazolone can be synthesized based upon the SAR of this project work and evaluated for pharmacological activity.

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