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Synthesis and Cardioprotective Activity of a Novel Series of Benzoxazole Derivatives

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Abstract: Novel series of benzoxazole derivatives were prepared by the condensation of methyl-2-(2-aminothiazol-5-ylamino) benzo[d]oxazole-5-carboxylate with various aromatic aldehydes. The structures of the synthesized compounds were VI_1-VI_{15} assigned on the basis of elemental analysis, IR, ^1H NMR and mass spectroscopy. These compounds were also screened for cardioprotective activity against Doxorubicin induced cardiotoxicity in rats. Selected two compounds produced a dose dependent cardioprotective activity by decreasing the doxorubicin elevated parameters like plasma Aspartate aminotransferase (AST), Creatinine kinase (CK-MB), Lactic acid dehydrogenase (LDH) and Triglyceride (TG) levels.

Keywords: Benzoxazole, Cardioprotective activity, Doxorubicin, Aspartate aminotransferase, Creatinine kinase, Lactic acid dehydrogenase, Triglyceride

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Introduction

It has been reported that substituted benzoxazoles and related heterocycles possess potential activity with lower toxicities in the chemotherapeutic approach in man (Haugwitz, *et al*., 1982; Hisano *et al*., 1982). Cautious literature survey exposed that targets containing benzoxazole moiety, either isolated from plants or accessed by total synthesis, have remarkable biological activities (Tipparaju et al., 2008) e.g. antimicrobial (Ilkay *et al*., 1998) Antihistaminic (Sener *et al*., 2018), antiparasitics (Albert *et al*., 2009), herbicidal (Diego *et al*., 2019), antiviral (Medebielle *et al*., 2008), anti-inflammatory (Aruna Devi *et al*., 2014), and antihelmintic (Satyendra *et al*., 2011) activities. Derivatives of thiazole have antibacterial (Khalil *et al*., 2009), antitubercular (Mahendra *et al*., 2009), anticonvulsant activity (Bachir *et al*., 1990) and anticancer (Elif *et al*., 2007) properties. In the present study, the thiazole moiety was connected to the benzoxazole moiety 2-position, (VI_1-VI_{15})

(Table. I), to combine different pharmacophores on one scaffold. Due to broad spectrum of activities reported in the literature so far, we herein report the synthesis of a novel series of methyl-2-(2-(benzylideneamino) thiazole-4-ylamino) benzoxazole-5-carboxylate derivatives (VI_1-VI_{15}) as the target compounds in order to examine their Cardio protective activity.

Myocardial infarction (MI), colloquially known as "heart attack," is caused by decreased or complete cessation of blood flow to a portion of the myocardium. Myocardial infarction may be "silent" and go undetected, or it could be a catastrophic event leading to hemodynamic deterioration and sudden death (Thygesen *et al*., 2007). Most myocardial infarctions are due to underlying coronary artery disease, the leading cause of death in the United States. With coronary artery occlusion, the myocardium is deprived of oxygen. Prolonged deprivation of oxygen supply to the myocardium can lead to myocardial cell death and necrosis (Reimer *et al*., 1983). Patients can present with chest discomfort or pressure that can radiate to the neck, jaw, shoulder, or arm. In addition to the history and physical exam, myocardial ischemia may be associated with ECG changes and elevated biochemical markers such as cardiac troponins (Apple *et al*., 2017). So ischemia, if left untreated for a sufficient period of time, can cause damage or death (infarction) of heart muscle tissue. Consequences of Myocardial infarction include hyperlipidemia, peroxidation of membrane lipids and loss of plasma membrane integrity. Currently numbers of drugs are available to limit the extent of myocardial damage or to prevent myocardium from necrosis. But still there is need of synthetic compounds for management of Myocardial infarction. The present study was aimed to synthesize new benzaxozole derivative for the evaluation of cardioprotective activity against doxorubicin induced cardiotoxicity in rats.

Materials and Methods

The melting points were determined by a digital melting point apparatus and were uncorrected. IR spectra were recorded on a Perkin–Elmer 377 spectrophotometer, ^{1}H NMR spectra were measured on Bruker AV 400 MHz using DMSO as a solvent and TMS as an internal standard.

Drug and chemicals:

Doxorubicin (Cadila pharamceuticals, Hyd, India), Creatine kinase kit, Lactate dehydrogenase kit, Triglyceride kit (Erba Mannheim, Daman, India), Glutamic Oxaloacetic Transaminase Kit (Coral clinical systems, Verna, Goa, India).

Synthesis of Methyl-3-nitro-4-hydroxybenzoate (*I*):

To a solution of aluminium nitrate (40 g) in acetic acid- acetic anhydride (1:1) mixture (160 ml), was added an appropriate phenol (40 g) in small portions, while cooling and shaking occasionally. The reaction mixture was left at room temperature for 1.5 h while shaking the contents intermittently to complete the nitration. The resulting brown solution was diluted to complete the nitration. The resulting brown solution was diluted with ice-cold water and acidified with concentrated nitric acid to get a bulky, yellow precipitate. It was filtered, washed with small quantity of methanol and purified by recrystallization from alcohol to get a yellow crystalline solid (44 g, 85%), m.p. is73℃.

Synthesis of Methyl-3-amino-4-hydroxybenzoate (*II*):

4-carbomethoxy-2-nitrophenol (I, 10 g) was dissolved in boiling alcohol (50%, 100 ml) and sodium dithionite was added to this boiling alcohol solution until it becomes almost colorless. Then the alcohol was reduced to one-third of its volume by distillation and the residual liquid was triturated with crushed ice. The resulting colorless, shiny product was filtered, and dried in the air. Its purification was effected by recrystallization from benzene to get colorless, shiny scales (5.1 g; 60%) m.p. is 143℃.

Synthesis of methyl-2-(*2-chloroacetamido*) *benzoxazole-5-carboxylate* (*III*):

4-carbomethoxy-2-aminophenol (II, 1.3 mol) was dissolved in 1l methyl alcohol and cooled the solution to 5^{0}C by adding chopped ice. A cold

suspension of cyanogenbromide (1.5 mol) in 1l of water was added over a period of 5 min with rapid stirring. The reaction mixture was stirred for 45 min at room temperature, solid sodium bicarbonate (1.3 mol) in small portions over a period of 1.5 h was added to bring the pH 6.5 -7.0. Stirring was continued for another 1h. The solid was separated by filtration, washed with cold water and on recrystallization from ethyl alcohol has resulted white solid, yield 70% and m.p. is 238°C.

Synthesis of methyl 2-(2-chloroacetamido) benzo [d]oxazole-5-carboxylate (IV):

A mixture of methyl-2-aminobenzoxazole-5-carboxylate (III, 0.01mol) and chloroacetyl chloride (0.01mol) was taken in 20 ml of dry benzene and the reaction mixture was refluxed for 5 h on a water bath. The solvent was evaporated and the residue was washed first with benzene and then with Petroleum ether. The compound was recrystallized from suitable solvent(s). The compound was found to be containing yield 72% and m.p. is 177°C.

Microwave synthesis of methyl-2-(2-aminothiazol-5-ylamino)benzo[d]oxazole-5-carboxylate (V):

Methyl-2-(2-chloroacetamido) benzo[d]oxazole-5-carboxylate (IV, 0.01 mol) and thiourea (0.01 mol) were dissolved in 10 ml of absolute alcohol in conical flask. The conical flask was hanged with a funnel and was subjected to microwave irradiation at 480 Watts for 5min in LG-Microwave oven. The reaction was monitored by TLC. After the completion of the reaction the contents were cooled and triturated with crushed ice; the separated solid was filtered, washed with 1% $NaHCO_3$ solution and purified by recrystallization from ethanol and water mixture found to be containing yield 97% and m.p. is 199°C.

The IR Spectrum (KBr) of the compound exhibited characteristic absorption bands (cm^{-1}) at: 3450 (NH_2), 3146 (NH), 1672 (C=O), 1626(C=C), 1528 (C=N), 1342 (C-O-C), 1142(C=S).

PMR spectrum (DMSO-d_6) of the compound has been found to exhibit proton signals (δ ppm) at: 8.3(s, 1H, Ar-H), 7.8 (d, 1H, Ar-H), 7.6 (d, 1H, Ar-H), 7.0 (s, 1H, CH, thiazole ring), 6.3 (s, 2H, NH_2), 5.5 (s, 1H, NH), 3.9 (s, 3H, CH_3).

Microwave synthesis of Methyl-2-(2-(aryylideneamino)thiazol-5-ylaminobenzo[d] oxazole-5-carboxylates (VI1-15):

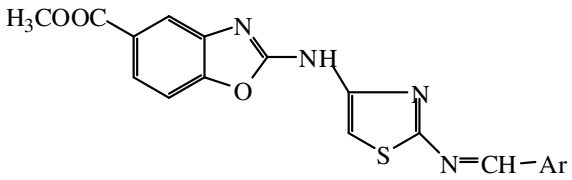
Methyl-2-(2-aminothiazol-4-ylamino)benzoxazole -5-carboxylate (V, 0.01 mol) and appropriate aromatic aldehydes viz. 4-dimethylaminophenyl, *4-t*-butylphenyl, Anisyl, phenyl, 4-hydroxy-phenyl,4-nitrophenyl, Veratryl, Cinnamyl, 3,4,5-trimethylphenyl, 4-tolyl, 2-hydroxyphenyl, 4-bromophenyl, 4-chlorophenyl, 2-naphthyl, 1-naphthyl (0.015 mol) were taken into a conical flask and were dissolved in 10 ml of absolute alcohol. The conical flask was hanged with a funnel and was subjected to microwave irradiation at 480 Watts for 7min in LG-Microwave oven. The reaction was monitored by TLC. The reaction mixture was cooled and triturated with crushed ice; the separated solid was filtered, washed with 1% $NaHCO_3$ solution and purified by recrystallization from ethanol and water mixture. The compounds were characterized by spectral data (Table 1).

Compound VI 1: Methyl-2-(2-(4-(dimethylamino) benzylideneaminothiazol-5-ylamino)benzo[d] oxazole-5-carboxylate:

Methyl- 2- (2- aminothiazol-5-ylamino)benzo[d] oxazole-5-carboxylate (V, 0.01 mol) and 4-dimethylaminophenyl (0.015 mol) were taken into a conical flask and were dissolved in 10 ml of absolute alcohol. The conical flask was hanged with a funnel and was subjected to microwave irradiation at 480 Watts for 7 min in LG-Microwave oven. The reaction mixture was cooled and triturated with crushed ice; the separated solid was filtered, washed with 1% $NaHCO_3$ solution and purified by recrystallization from ethanol and water mixture.

^1H NMR (DMSO-d_6): δ 8.8 (s, 1H, ArH), 8.2(s, 1H, CH), 8.1 (d, 1H,ArH), 8.0 (d, 1H, ArH),7.5 (d, 2H, ArH), 6.8(d, 2H, ArH), 6.3 (s, 1H, ArH, thiazole ring), 5.2 (s, 1H, NH), 3.9 (s, 3H, CH_3), 3.0 (s, 6H, $(CH_2)_3$).

Table1: Physical data of methyl 2-(2-(benzylideneamino) thiazol-4-ylamino) benzoxazole-5-carboxylates (VI)



Compd	Ar	Molecular Formula (VI)	Melting Point (°C)	Yield (%)
AS1	4-dimethylamino phenyl	$C_{21}H_{19}N_5O_3S$	208	90
AS2	*4-t*-butylphenyl	$C_{23}H_{22}N_4O_3S$	301	93
AS3	4-methoxyphenyl	$C_{20}H_{16}N_4O_4S$	280	98
AS4	Phenyl	$C_{19}H_{14}N_4O_3S$	201	92
AS5	**4-hydroxy phenyl**	$C_{19}H_{14}N_4O_4S$	228	95
AS6	4-nitrophenyl	$C_{19}H_{13}N_5O_5S$	299	97
AS7	3,4 dimethoxyphenyl	$C_{19}H_{18}N_4O_5S$	226	96
AS8	Cinnamyl	$C_{21}H_{15}N_4O_3S$	240	94
AS9	3,4,5-trimethylphenyl	$C_{22}H_{20}N_4O_3S$	238	95
AS10	4-methylphenyl	$C_{20}H_{16}N_4O_3S$	303	91
AS11	2-hydroxyphenyl	$C_{19}H_{14}N_4O_4S$	234	91
AS12	4-bromophenyl	$C_{19}H_{13}BrN_4O_3S$	305	90
AS13	**4-chlorophenyl**	$C_{19}H_{13}ClN_4O_3S$	222	99
AS14	2-naphthyl	$C_{23}H_{16}N_4O_3S$	341	90
AS15	1-naphthyl	$C_{23}H_{16}N_4O_3S$	322	91

IR (KBr) CM^{-1}: 3096 (NH), 1683 (C=O), 1640(C=C), 1576 (C=N), 1442(C-O-C), 1383(C=S), MS (*m/z):* M^+: 422.1, *Anal.* Calcd for $C_{21}H_{19}N_5O_3S$: C, 59.84; H, 4.54; N, 16.62; O, 11.39; S, 7.61.

Compound VI 2: methyl-2-(2-(4-tert-butylbenzyl ideneamino)thiazol-5-ylamino) benzo[d]oxazole-5-carboxylate:

Methyl-2-(2-aminothiazol-5-ylamino)benzo[d]oxazole-5-carboxylate (V, 0.01 mol) and 4-*t*-butylbenzaldehyde (0.015 mol) were taken into a conical flask and were dissolved in 10ml of absolute alcohol. The conical flask was hanged with a funnel and was subjected to microwave irradiation at 480 Watts for 7 min in LG-Microwave oven. The reaction mixture was cooled and triturated with crushed ice; the separated solid was filtered, washed with 1% $NaHCO_3$ solution and purified by recrystallization from ethanol and water mixture.

^1H NMR (DMSO-d_6): δ 8.7 (s, 1H, ArH), 8.2(s, 1H, CH), 8.1 (d, 1H,ArH), 8.0 (d, 1H, ArH),7.5 (d, 2H, ArH), 7.1(d, 2H, ArH), 6.0 (s, 1H, ArH, thiazole ring), 5.4 (s, 1H, NH), 3.9 (s, 3H, CH_3), 1.3(s, 9H, $(CH_3)_3$).

IR (KBr) CM^{-1}: 3091 (NH), 1681 (C=O), 1642 (C=C), 1577 (C=N), 1443 (C-O-C), 1381 (C=S), MS (*m/z):* M^+: 435.1, *Anal.* Calcd for $C_{23}H_{22}N_4O_3S$: C, 63.58; H, 5.10; N, 12.89; O, 11.05; S, 7.38.

Compound VI 3: methyl-2-(2-(4-methoxybenzyl ideneamino)thiazol-5-ylamino)benzo[d]oxazole-5-carboxylate:

Methyl- 2- (2- aminothiazol-5-ylamino) benzo[d] oxazole-5-carboxylate (V, 0.01 mol) and 4-methoxybutylbenzaldehyde (0.015 mol) were taken into a conical flask and were dissolved in 10 ml of absolute alcohol. The conical flask was hanged with a funnel and was subjected to microwave irradiation at 480 Watts for 7 min in LG-Microwave oven. The reaction mixture was cooled and triturated with crushed ice; the separated solid was filtered, washed with 1% $NaHCO_3$ solution and purified by recrystallization from ethanol and water mixture.

^{1}H NMR (DMSO-d_6): δ 8.9 (s, 1H, CH), 8.7 (s, 1H, CH), 8.1 (d, 1H,ArH), 8.0(d, 1H, ArH),7.8 (d, 2H, ArH), 7.1 (d, 2H, ArH), 6.2 (s, 1H, ArH, thiazole ring), 5.0 (s, 1H, NH), 3.9 (s, 3H, CH_3), 3.8 (s, 3H, OCH_3).

IR (KBr) CM^{-1}: 3068 (NH), 1687 (C=O), 1645 (C=C), 1512 (C=N), 1432 (C-O-C), 1371 (C=S), MS (*m/z):* M^+: 409.0, *Anal.* Calcd for $C_{20}H_{16}N_4O_4S$: C, 58.81; H, 3.95; N, 13.72; O, 15.67; S, 7.85.

Compound VI 4: methyl-2-(2-(benzylideneamino) thiazol-5-ylamino)benzo[d]oxazole-5-carboxylate:

Methyl- 2- (2- aminothiazol-5-ylamino) benzo[d] oxazole-5-carboxylate (V, 0.01 mol) and benzaldehyde (0.015 mol) were taken into a conical flask and were dissolved in 10 ml of absolute alcohol. The conical flask was hanged with a funnel and was subjected to microwave irradiation at 480 Watts for 7 min in LG-Microwave oven. The reaction mixture was cooled and triturated with crushed ice; the separated solid was filtered, washed with 1% $NaHCO_3$ solution and purified by recrystallization from ethanol and water mixture.

^{1}H NMR (DMSO-d_6): δ 8.6 (s, 1H, ArH), 8.1(s, 1H, CH), 8.0 (d, 1H,ArH), 7.9 (d, 1H, ArH),7.5 (d, 2H, ArH), 7.0(t, 3H, ArH), 6.1 (s, 1H, ArH, thiazole ring), 5.4 (s, 1H, NH), 3.8 (s, 3H, CH_3).

IR (KBr) CM^{-1}: 3076 (NH), 1679 (C=O), 1646(C=C), 1580 (C=N), 1448(C-O-C), 1373 (C=S), MS (*m/z):* M^+: 379.0, *Anal.* Calcd for $C_{19}H_{14}N_4O_3S$: C, 60.31; H, 3.73; N, 14.81; O, 12.68; S, 8.47.

Compound VI 5: methyl-2-(2-(4-hydroxybenzyl ideneamino)thiazol-5-ylamino)benzo[d]oxazole-5-carboxylate:

Methyl- 2- (2- aminothiazol-5-ylamino) benzo[d] oxazole-5-carboxylate (V, 0.01 mol) and 4-hydroxy benzaldehyde (0.015 mol) were taken into a conical flask and were dissolved in 10ml of absolute alcohol. The conical flask was hanged with a funnel and was subjected to microwave irradiation at 480 Watts for 7 min in LG-Microwave oven. The reaction mixture was cooled and triturated with crushed ice; the separated solid was filtered, washed with 1% $NaHCO_3$ solution and purified by recrystallization from ethanol and water mixture.

^{1}H NMR (DMSO-d_6): δ9.2 (S,1H,OH), 8.9 (s, 1H, ArH), 8.5 (s, 1H, CH), 8.1 (d, 1H,ArH), 8.0(d, 1H, ArH),7.7 (d, 2H, ArH), 6.8(d, 2H, ArH), 6.4 (s, 1H, ArH, thiazole ring), 4.6(s, 1H, NH), 3.7 (s, 3H, CH_3).

IR (KBr) CM^{-1}: 3091 (NH), 1699 (C=O), 1676 (C=C), 1580 (C=N), 1455 (C-O-C), 1371 (C=S), MS (*m/z):* M^+: 395.0, *Anal.* Calcd for $C_{19}H_{14}N_4O_4S$: C, 57.86; H, 3.58; N, 14.21; O, 16.23; S, 8.13.

Compound VI 6: methyl-2-(2-(4-nitrobenzyl ideneamino)thiazol-5-ylamino)benzo[d]oxazole-5-carboxylate:

Methyl - 2- (2- aminothiazol-5-ylamino)benzo[d] oxazole-5-carboxylate (V, 0.01 mol) and 4-nitro benzaldehyde (0.015 mol) were taken into a conical flask and were dissolved in 10 ml of absolute alcohol. The conical flask was hanged with a funnel and was subjected to microwave irradiation at 480 Watts for 7 min in LG-Microwave oven. The reaction mixture was cooled and triturated with crushed ice; the separated solid was filtered, washed with 1% $NaHCO_3$ solution and purified by recrystallization from ethanol and water mixture.

^{1}H NMR (DMSO-d_6): δ 8.6 (s, 1H, CH), 8.4 (s, 1H,ArH), 8.3 (d, 2H, ArH),8.1 (d, 1H, ArH), 7.9 (d, 1H, ArH), 7.8 (d, 2H, ArH), 6.5 (s, 1H, ArH, thiazole ring), 5.5 (s, 1H, NH), 3.8 (s, 3H, OCH_3).

IR (KBr) CM^{-1}: 3091 (NH), 1692 (C=O), 1684 (C=C), 1582 (C=N), 1449 (C-O-C), 1373 (C=S), MS (*m/z):* M^+: 424.0, *Anal.* Calcd for $C_{19}H_{13}N_5O_5S$: C, 53.90; H, 3.09; N, 16.54; O, 18.89; S, 7.57.

Compound VI 7: methyl-2-(2-(3,4-dimethoxybenzyl ideneamino)thiazol-5-ylamino)benzo[d]oxazole-5-carboxylate:

Methyl – 2 -(2 -aminothiazol-5-ylamino)benzo[d] oxazole-5-carboxylate (V, 0.01 mol) and 3, 4-dimethoxy benzaldehyde (0.015 mol) were taken into a conical flask and were dissolved in 10 ml of absolute alcohol. The conical flask was hanged with a funnel and was subjected to microwave irradiation at 480 Watts for 7 min in LG-

Microwave oven. The reaction mixture was cooled and triturated with crushed ice; the separated solid was filtered, washed with 1% $NaHCO_3$ solution and purified by recrystallization from ethanol and water mixture.

^1H NMR (DMSO-d_6): δ 8.9 (s, 1H, ArH), 8.5 (s, 1H, CH), 8.1 (d, 1H,ArH), 8.0(d, 1H, ArH),7.6 (s, 1H, ArH), 7.4 (d, 1H, ArH), 6.9 (d, 1H, ArH), 6.5 (s, 1H, ArH, thiazole ring), 4.8 (s, 1H, NH), 3.8 (s, 9H, $3OCH_3$).

IR (KBr) CM^{-1}: 3071 (NH), 1687 (C=O), 1663 (C=C), 1575 (C=N), 1451 (C-O-C), 1373 (C=S) , S (*m/z):* M^+: 439.0, *Anal.* Calcd for $C_{21}H_{18}N_4O_5S$: C, 57.53; H, 4.14; N, 12.78; O, 18.25; S, 7.31.

Compound VI 8: methyl-2-(2-(3-phenylallyl ideneamino)thiazol-5-ylamino)benzo[d]oxazole-5-carboxylate:

Methyl – 2 - (2 - aminothiazol-5-ylamino)benzo[d] oxazole-5-carboxylate (V, 0.01 mol) and cinnamaldehyde (0.015 mol) were taken into a conical flask and were dissolved in 10 ml of absolute alcohol. The conical flask was hanged with a funnel and was subjected to microwave irradiation at 480 Watts for 7 min in LG-Microwave oven. The reaction mixture was cooled and triturated with crushed ice; the separated solid was filtered, washed with 1% $NaHCO_3$ solution and purified by recrystallization from ethanol and water mixture.

^1H NMR (DMSO-d_6): δ 8.8 (s, 1H, ArH), 8.1 (d, 1H,ArH), 8.0(d, 1H, ArH),7.6 (t, 2H, ArH), 7.5 (s, 1H, CH), 7.4 (t, 2H, ArH), 7.3(t, 1H, ArH),7.0 (s, 1H, CH), 6.4 (s, 1H, ArH, thiazole ring), 5.6 (s, 1H, CH), 4.2 (s, 1H, NH), 3.7 (s, 3H, OCH_3).

IR (KBr) CM^{-1}: 3088 (NH), 1681 (C=O), 1667 (C=C), 1573 (C=N), 1455 (C-O-C), 1371 (C=S), MS (*m/z):* M^+: 405.1, *Anal.* Calcd for $C_{21}H_{16}N_4O_3S$: C, 62.36; H, 3.99; N, 13.85; O, 11.87; S, 7.93.

Compound VI 9: methyl-2-(2-(3,4,5-trimethylbenzyl ideneamino)thiazol-5-ylamino)benzo[d]oxazole-5-carboxylate:

Methyl – 2 - (2- aminothiazol-5-ylamino)benzo[d] oxazole-5-carboxylate (V, 0.01 mol) and 3,4,5-trimethyl benzaldehyde (0.015 mol) were taken into a conical flask and were dissolved in 10ml of absolute alcohol. The conical flask was hanged with a funnel and was subjected to microwave irradiation at 480 Watts for 7 min in LG-Microwave oven. The reaction mixture was cooled and triturated with crushed ice; the separated solid was filtered, washed with 1% $NaHCO_3$ solution and purified by recrystallization from ethanol and water mixture.

^1H NMR (DMSO-d_6): δ 8.7 (s, 1H, ArH), 8.6 (s, 1H,CH), 8.1 (d, 1H, ArH),8.0 (d, 1H, ArH), 7.3 (d, 2H, ArH), 6.1 (s, 1H, ArH, thiazole ring), 4.8 (s, 1H, NH), 3.8 (s, 3H, OCH_3), 2.3 (s, 6H, $2CH_3$), 2.1 (s, 1H, CH_3).

IR (KBr) CM^{-1}: 3090 (NH), 1674 (C=O), 1660 (C=C), 1569 (C=N), 1449 (C-O-C), 1367 (C=S), MS (*m/z):* M^+: 421.1, *Anal.* Calcd for $C_{22}H_{20}N_4O_3S$: C, 62.84; H, 4.79; N, 13.32; O, 11.41; S, 7.63.

Compound VI 10: methyl-2-(2-(4-methylbenzyl ideneamino)thiazol-5-ylamino)benzo[d]oxazole-5-carboxylate:

Methyl- 2- (2- aminothiazol-5-ylamino) benzo[d] oxazole-5-carboxylate (V, 0.01 mol) and 4-methyl benzaldehyde (0.015 mol) were taken into a conical flask and were dissolved in 10ml of absolute alcohol. The conical flask was hanged with a funnel and was subjected to microwave irradiation at 480 Watts for 7 min in LG-Microwave oven. The reaction mixture was cooled and triturated with crushed ice; the separated solid was filtered, washed with 1% $NaHCO_3$ solution and purified by recrystallization from ethanol and water mixture.

^1H NMR (DMSO-d_6): δ 8.8 (s, 1H, CH), 8.6 (s, 1H,ArH), 8.1 (d, 1H, ArH),8.0 (d, 1H, ArH), 7.7 (d, 2H, ArH), 7.2 (d, 2H, ArH), 6.3 (s, 1H, ArH, thiazole ring), 5.1 (s, 1H, NH), 4.0(s, 3H, OCH_3), 2.3 (s, 3H, CH_3).

IR (KBr) CM^{-1}: 3092 (NH), 1695 (C=O), 1689 (C=C), 1589 (C=N), 1458 (C-O-C), 1371 (C=S), MS (*m/z):* M^+:393.1, *Anal.* Calcd for $C_{20}H_{16}N_4O_3S$: C, 61.21; H, 4.11; N, 14.28; O, 12.23; S, 8.17.

Compound VI 11: methyl-2-(2-(2-hydroxybenzyl ideneamino)thiazol-5-ylamino)benzo[d]oxazole-5-carboxylate:

Methyl- 2- (2- aminothiazol-5-ylamino) benzo[d] oxazole-5-carboxylate (V, 0.01 mol) and 2-hydroxy benzaldehyde (0.015 mol) were taken into a conical flask and were dissolved in 10 ml of absolute alcohol. The conical flask was hanged with a funnel and was subjected to microwave irradiation at 480 Watts for 7 min in LG-Microwave oven. The reaction mixture was cooled and triturated with crushed ice; the separated solid was filtered, washed with 1% $NaHCO_3$ solution and purified by recrystallization from ethanol and water mixture.

^1H NMR (DMSO-d_6): δ 11.2 (S,1H,OH), 8.9 (s, 1H, ArH), 8.6 (s, 1H, CH), 8.1 (d, 1H,ArH), 8.0(d, 1H, ArH),7.6 (d, 1H, ArH), 7.5 (t, 1H, ArH), 7.2 (t, 1H, ArH), 7.0 (t, 1H, ArH), 6.3 (s, 1H, ArH, thiazole ring), 5.0 (s, 1H, NH), 3.9 (s,3H,CH_3).

IR (KBr) CM^{-1}: 3088 (NH), 1697 (C=O), 1666 (C=C), 1578 (C=N), 1454 (C-O-C), 1375 (C=S), MS (*m/z):* M^+: 395.0, *Anal.* Calcd for $C_{19}H_{14}N_4O_4S$: C, 57.86; H, 3.58; N, 14.21; O, 16.23; S, 8.13.

Compound VI 12: methyl-2-(2-(4-bromobenzyl ideneamino)thiazol-5-ylamino)benzo[d]oxazole-5-carboxylate:

Methyl- 2- (2- aminothiazol-5-ylamino) benzo[d] oxazole-5-carboxylate (V, 0.01 mol) and 4-bromo benzaldehyde (0.015 mol) were taken into a conical flask and were dissolved in 10 ml of absolute alcohol. The conical flask was hanged with a funnel and was subjected to microwave irradiation at 480 Watts for 7 min in LG-Microwave oven. The reaction mixture was cooled and triturated with crushed ice; the separated solid was filtered, washed with 1% $NaHCO_3$ solution and purified by recrystallization from ethanol and water mixture.

^1H NMR (DMSO-d_6): δ 8.7 (s, 1H, CH), 8.5 (s, 1H, CH), 8.0 (d, 1H,ArH), 7.9(d, 1H, ArH),7.6 (d, 1H, ArH), 7.5 (d, 2H, ArH), 7.3 (d, 2H, ArH), 5.9 (s, 1H, ArH, thiazole ring), 5.0 (s, 1H, NH), 3.9 (s, 3H, CH_3).

IR (KBr) CM^{-1}: 3093 (NH), 1693 (C=O), 1679 (C=C), 1583 (C=N), 1446 (C-O-C), 1376 (C=S), MS (*m/z):* M^+: 458.1, *Anal.* Calcd for $C_{19}H_{13}BrN_4O_3S$: C, 49.90; H, 2.87; Br, 17.47; N, 12.25; O, 10.50; S, 7.01.

Compound VI 13: methyl-2-(2-(2-chlorobenzyl ideneamino)thiazol-5-ylamino)benzo[d]oxazole-5-carboxylate:

Methyl- 2- (2- aminothiazol-5-ylamino) benzo[d] oxazole-5-carboxylate (V, 0.01 mol) and 4-chloro benzaldehyde (0.015mol) were taken into a conical flask and were dissolved in 10 ml of absolute alcohol. The conical flask was hanged with a funnel and was subjected to microwave irradiation at 480 Watts for 7 min in LG-Microwave oven. The reaction mixture was cooled and triturated with crushed ice; the separated solid was filtered, washed with 1% $NaHCO_3$ solution and purified by recrystallization from ethanol and water mixture.

^1H NMR (DMSO-d_6): δ 8.8 (s, 1H, CH), 8.6 (s, 1H, CH), 8.1 (d, 1H,ArH), 8.0(d, 1H, ArH),7.7 (d, 1H, ArH), 7.5 (d, 1H, ArH), 7.4 (t, 1H, ArH), 7.3 (t, 1H, ArH), 6.4 (s, 1H, ArH, thiazole ring), 5.2 (s, 1H, NH), 3.6 (s, 3H, CH_3).

IR (KBr) CM^{-1}: 3094 (NH), 1691 (C=O), 1680 (C=C), 1581 (C=N), 1447 (C-O-C), 1374 (C=S), MS (*m/z):* M^+: 413.0, , *Anal.* Calcd for $C_{19}H_{13}ClN_4O_3S$: C, 55.28; H, 3.17; Cl, 8.59; N, 13.57; O, 11.63; S, 7.77.

Compound VI 14: methyl-2-(2-(naphthalen-2-ylmethyleneamino)thiazol-5-ylamino)benzo[d] oxazole-5-carboxylate:

Methyl- 2- (2- aminothiazol-5-ylamino) benzo[d] oxazole-5-carboxylate (V, 0.01 mol) and 2-naphthaldehyde (0.015 mol) were taken into a conical flask and were dissolved in 10 ml of absolute alcohol. The conical flask was hanged with a funnel and was subjected to microwave irradiation at 480 Watts for 7 min in LG-Microwave oven. The reaction mixture was cooled and triturated with crushed ice; the separated solid was filtered, washed with 1% $NaHCO_3$

solution and purified by recrystallization from ethanol and water mixture.

^{1}H NMR (DMSO-d_6): δ 8.7 (s, 1H, CH), 8.5 (s, 1H, CH), 8.4 (d, 1H,ArH), 8.3 (t, 1H, ArH),8.2 (d, 1H, ArH), 8.1(d, 1H, ArH), 8.0(d,1H, ArH),7.9(d, 1H, ArH),7.7(t, 1H, ArH), 7.4 (t, 1H, ArH), 6.3 (s, 1H, ArH, thiazole ring), 4.1 (s, 1H, NH), 3.9 (s, 3H, OCH_3).

IR (KBr) CM^{-1}: 3090 (NH), 1692 (C=O), 1683 (C=C), 1585 (C=N), 1453 (C-O-C), 1370 (C=S), MS (*m/z):* M^+: 428.0, *Anal.* Calcd for $C_{23}H_{16}N_4O_3S$: C, 64.47; H, 3.76; N, 13.08; O, 11.20; S, 7.48.

Compound VI 15: methyl-2-(1-(naphthalen-2-ylmethyleneamino)thiazol-5-ylamino)benzo[d] oxazole-5-carboxylate:

Methyl - 2- (2- aminothiazol-5-ylamino)benzo[d] oxazole-5-carboxylate (V, 0.01 mol) and 1-naphthaldehyde (0.015mol) were taken into a conical flask and were dissolved in 10 ml of absolute alcohol. The conical flask was hanged with a funnel and was subjected to microwave irradiation at 480 Watts for 5-7 min in LG-Microwave oven. The reaction mixture was cooled and triturated with crushed ice; the separated solid was filtered, washed with 1% $NaHCO_3$ solution and purified by recrystallization from ethanol and water mixture.

^{1}H NMR (DMSO-d_6): δ 8.8 (s, 1H, CH), 8.5 (s,1H, CH), 8.6 (d, 1H,ArH), 8.2 (t, 1H, ArH),8.0 (d, 1H, ArH),7.9(d,1H, ArH), 7.8(d,1H, ArH), 7.7 (d, 1H, ArH),7.6 (t, 1H, ArH), 7.5 (t, 1H, ArH), 6.1 (s, 1H, ArH, thiazole ring), 4.8 (s,1H, NH),3.4 (s, 3H, OCH_3).

IR (KBr) CM^{-1}: 3093 (NH), 1689 (C=O), 1687 (C=C), 1581 (C=N), 1458 (C-O-C), 1365 (C=S), MS (*m/z):* M^+: 428.0, *Anal.* Calcd for $C_{23}H_{16}N_4O_3S$: C, 63.74; H, 5.17; N, 12.04; O, 12.20; S, 6.85.

Evaluation of Test compounds for Cardioprotective activity:

Among all the synthesized compounds, the compounds coded as AS1 and AS2 were selected for further screening of cardioprotective activity in rats.

Experimental animals:

36 female Wistar rats (180-200 g) were obtained from Mahaveera Enterprises (Hyderabad, India). The animals were housed in cages under hygienic conditions and placed in a controlled environment (12 h light–dark cycle, 25±2 °C and 45±10% humidity) with free access to water and fed with standard diet *ad libitum*. Care was taken to avoid stressful conditions.

Study design:

Rats were randomly divided into 6 groups (n = 6) and treated as follow:

Group 1: received 0.5% CMC (orally) daily for 7 days and served as control.

Group 2: received 0.5% CMC (orally) daily for 7 days and on 6th day single dose of doxorubicin (15 mg/kg., i.p.) (Nagi and Mansour, 2000) and served as cardiotoxic control.

Group 3: received compound AR1 (50 mg/kg/day, orally) daily for 7 days and on 6th day single dose of doxorubicin (15 mg/kg., i.p.).

Group 4: received compound AR1 (100 mg/kg/day, orally) daily for 7 days and on 6th day single dose of doxorubicin (15 mg/kg., i.p.).

Group 5: received compound AR2 (100 mg/kg/day, orally) daily for 7 days and on 6th day single dose of doxorubicin (15mg/kg., i.p.).

Group 6: received compound AR2 (100 mg/kg/day, orally) daily for 7 days and on 6th day single dose of doxorubicin (15 mg/kg., i.p.) (Reddy *et al*., 2012).

On the 7th day of the experiment, blood samples were collected through retro-orbital puncture method. After centrifugation, plasma was separated and stored at -20°C for biochemical analysis.

Analysis of blood samples:

Plasma samples were analyzed for cardiotoxic biomarkers like Aspartate amino transferase (AST), lactic acid dehydrogenase (LDH) and Creatine kinase (CK-MB) and also for plasma lipid

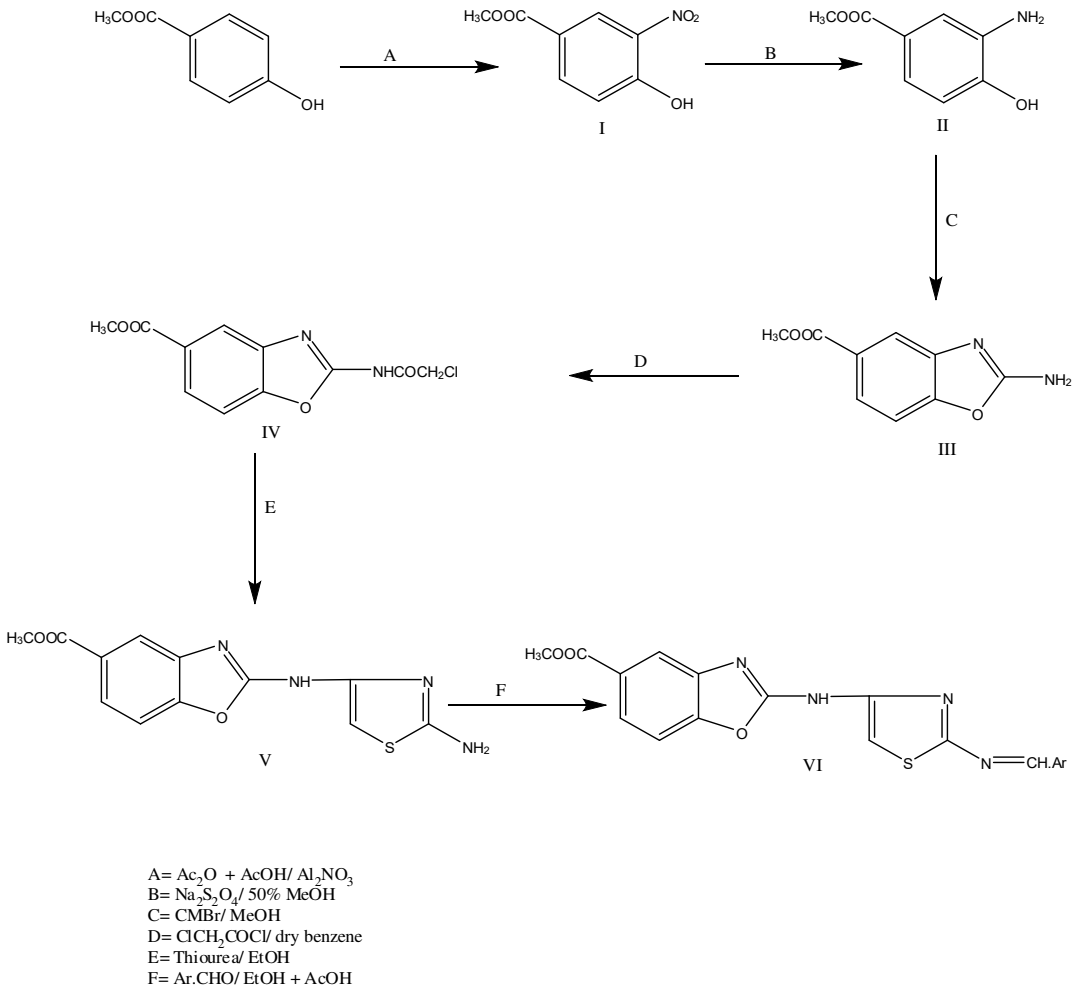


Fig. 1: Synthesis of target compounds.

levels, especially Triglyceride levels using commercially available diagnostic kits (George, 1975).

Statistical analysis:

All the values were expressed as Mean ±Standard deviation (S.D) (n=6). Statistical comparisons between different groups were done by using one way analysis of variance followed by Tuckey post test. $P < 0.05$ was considered as statistically significant.

Results and Discussion

The target compounds were synthesized according to Figure 1. The required starting material, methyl 3-amino-4-hydroxybenzoate (II) was prepared in good yield (85%). The starting material (II) on cyclization with cyanogen bromide in methyl alcohol on rapid stirring at room temperature gave the product, methyl 2-aminobenzo[d]oxazole-5-carboxylate (III). Methyl-2-aminobenzoxazole-5-carboxylate (III) on reaction with chloro acetyl chloride in dry benzene yields the compound, methyl-2-(2-chloroacetamido) benzo[d]oxazole-5-carboxylate (IV). methyl 2-(2-chloroacetamido) benzo[d] oxazole-5-carboxylate (IV) on cyclization with thiourea gave the compound methyl-2-(2-aminothiazol- 5 - ylamino) benzo [d] oxazole-5-carboxylate (V) which on reaction with various aromatic aldehydes viz, 4-dimethylaminophenyl, *4-t*-butylphenyl, Anisyl, phenyl, 4-hydroxy-phenyl,4-nitrophenyl, Veratryl , Cinnamyl, 3,4,5-trimethylphenyl, 4- tolyl, 2- hydroxyphenyl, 4-

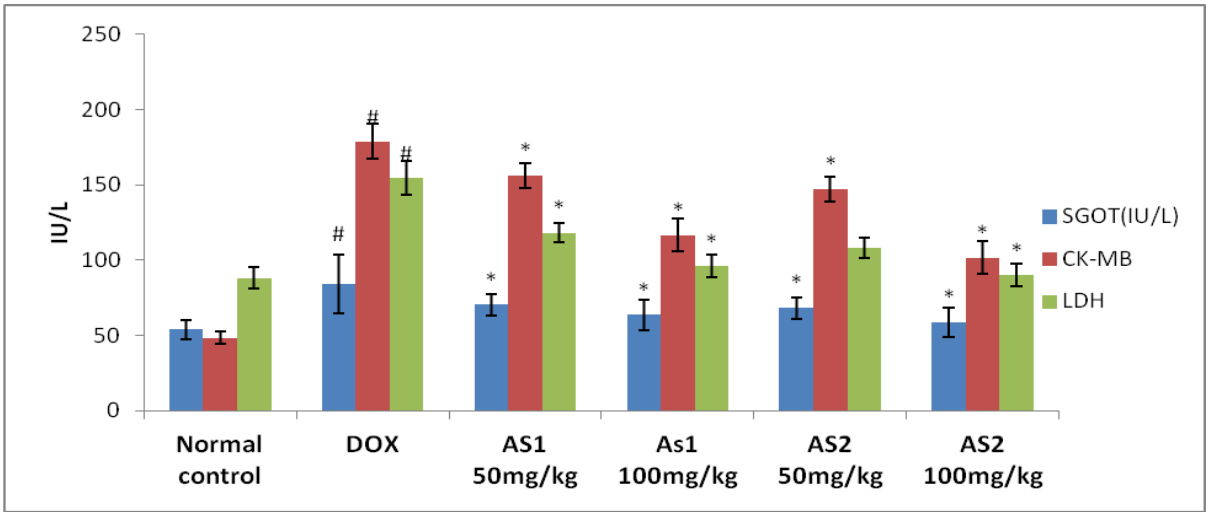


Fig. 2: Effect of test compounds on AST, CK-MB, LDH levels in rats treated with doxorubicin.

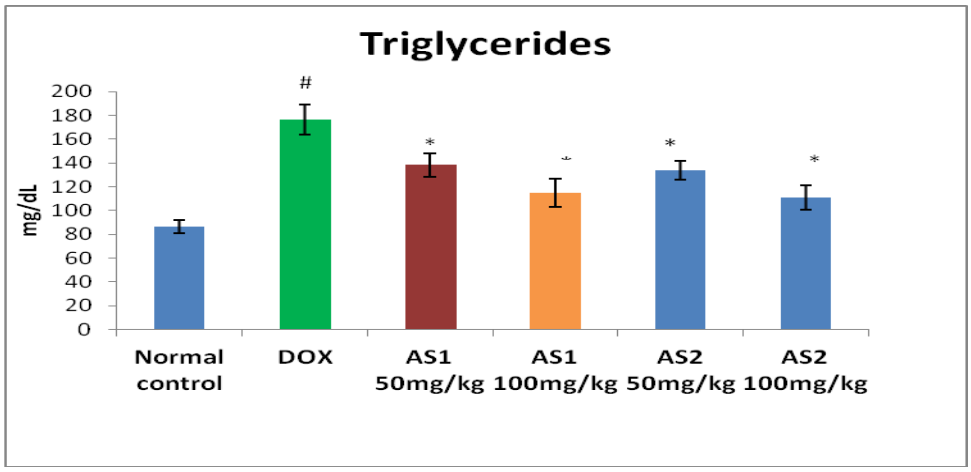


Fig 3: Effect of Test compounds on TG levels in rats treated with doxorubicin.

bromophenyl, 4-chlorophenyl, 2-naphthyl, 1-naphthyl conveniently converted into the targeted compounds Methyl -2 - (2- (aryylideneamino) thiazol-5-ylamino) benzo [d] oxazole – 5 - carboxylates (VI).

The yields, melting points and physical data of newly synthesized compounds are summarized in Table 1. Among synthesized fifteen compounds we have selected two compounds i.e. AS5 where the Ar group is 4-hydroxy phenyl and AS13 where the Ar group is 4-chloro phenyl for further investigations.

Cardioprotective activity:

The general appearance of all rats was observed every day after the treatment. No mortality was observed in all groups. Rats in the DOX alone treated group showed scruffy fur and developed a light yellow tinge. These animals appeared sicker, weaker and lethargic as compared to AS+DOX treated group. In this study, we evaluated the Cardioprotective effects of test compounds in rats by measuring plasma Aspartate aminotransferase (AST), Lactate dehydrogenase (LDH), Creatine kinase (CK-MB), Triglyceride (TG) and the results are shown in Figures 2 and 3.

In this study, plasma markers indicating myocardial injury like plasma AST, LDH, CK-MB and TG were significantly elevated ($P<0.001$) in Doxorubicin treatment group when compared

with normal control group (Figs. 2, 3). Pre-treatment with test compounds in AS+DOX groups at different doses (50 mg/kg/day and 10 mg/kg/day, respectively) almost restored the raised AST, CK-MB,LDH and TG levels when compared with DOX only treated group (P<0.001).

In the present study, Doxorubicin (15 mg/kg, i.p.) was used to induce cardiotoxicity. Evaluation of cardiotoxicity was done by measuring plasma AST, LDH, and CK enzyme activities, which are important measures of both early and late phases of cardiac injury (Saad *et al*., 2001). DOX Administration to rats significantly elevated plasma AST, LDH, CK-MB, TG levels (P<0.001) which are released from damaged myocytes and sensitive markers of cardiac injury (Reddy *et al*., 2012). Treatment with test compounds (AS1 and AS2) results in significant inhibition of DOX elevated plasma AST, LDH, TG and CK enzyme levels (Figs. 2,3). In conclusion, it was found based on the above findings that test compounds has produced a mild to moderate cardioprotective effect as evidenced by decreased cardiac injury markers in rats.

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