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Synthesis, Characterization and Cardioprotective Activity of Some Novel Benzotriazole and Pyrazole Derivatives

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A series of N-(1-(1*H*-benzo[d]) [1,2,3]triazol-1-yl)-2,2-dimethyl propyl)-2-(substituted phenyl) acetamide derivatives and methyl 5-((4-(2,5-disubstituted phenyl) furan-2 carboxylate derivatives are prepared from different substituted aryl carboxylic acids, phenyl acetic acid and cinnamic acid, respectively. All the synthesized compounds are investigated for cardioprotective activity by ischemia reperfusion method, while all the compounds show significant activity.

Key Words: Substituted benzotriazole, Pyrazoles, Aryl carboxylic acids, Cardioprotective.

INTRODUCTION

Benzotriazoles derivatives are reported as antiinflammatory[1], amide substituted benzotriazole derivitives[2] as inhibitors of poly(ADP-ribose) polymerase (PARP) and thus are useful for the treatment of cancer, inflammatory diseases, reperfusion injuries, ischemic conditions, stroke, renal failure, cardiovascular diseases, diabetes, neurode generative diseases. 2-(4-(Dialkyl amino alkoxy) phenyl) benzotriazoles and N-oxides are reported[3] as thromboxane A_2 antagonists and as hypocholesterolemic agents, platelet aggregation inhibitors. Some benzotriazole carboxylic acid or ester derivatives are reported[4] for prophylaxis and treatment of metabolic related disorders including atherosclerosis, coronary heart disease and type 2 diabetes, 5-(substituted) benzotriazoles and triazolyl benzotriazoles as potential potassium channel activators[5]. Pyrazolo derivatives are reported[6] in the literature as protein kinase inhibitors particularly in the treatment of diseases such as cancer, inflammatory disorders, restenosis and cardiovascular diseases, phenyl acetamido pyrazoles as antitumor agents[7]. Hence, in the present study some novel benzotriazole and pyrazole derivatives are synthesized and characterized by ^{1}H NMR, IR and mass spectroscopy. 2-(Substituted phenyl) acetic acid was converted into its amide derivative by treatment with thionyl chloride in ammonia gas[8,9] which then on condensation with 1*H*-benzotriazole[10,11] and trimethyl acetaldehyde gives compounds **1-5**. Methyl 5-(chloromethyl) furan-2-carboxylate was condensed with 4-nitro pyrazole to give an intermediate nitro compound

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which on reduction with palladium hydroxide in methanol gives corresponding amino compound the later on condensation with different **3**, **5** disubstituted cinnamic acids give compounds **6-10**. All the compounds are investigated for cardioprotective activity by ischemia reperfusion method while all the compounds show significant activity.

EXPERIMENTAL

All the melting points are uncorrected and were taken in open capillaries. The progress of reaction and purity of products was checked by TLC using silica gel-G and methanol in chloroform. IR spectrum was taken on Jasco's FTIR spectrometer using KBr pellet technique. ^{1}H and ^{13}C NMR spectra were recorded on Varian 400 MHz NMR spectrometer in $CDCl_3$ solvent. Mass spectra were recorded on Agilent MS ion trap systems.

General synthesis

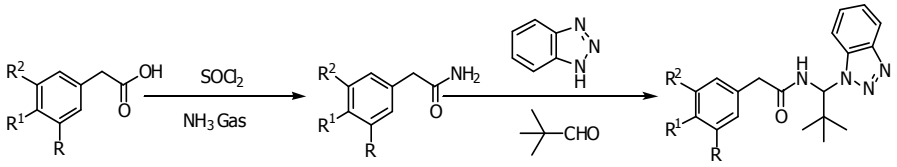
N-(1-(1*H*-Benzo[d][1,2,3]triazol-1-yl)-2,2-dimethylpropyl)-2-(3,4-dimethoxyphenyl)acetamide (1): Step-01: Preparation of 2-(3,4-dimethoxyphenyl)acetamide: 10 g of 2-(3,4-dimethoxyphenyl)acetic acid (1 eq) was dissolved in 100 mL of dichloromethane (5 times w/v). Added drop wise 12 g of thionyl chloride (2 eq) at ambient temperature. The reaction mixture was stirred till the reaction completion (about 2-4 h) and reaction was monitored by TLC (mobile phase for TLC 5 % methanol in chloroform). After completion of the reaction. The reaction mass was distilled under reduced pressure at below 45 °C and the residue was dissolved in 100 mL of tetrahydrofuran (5 times w/v) and passed ammonia gas till the desired compound formation (about 1-2 h) and the precipitated solid was collected by filtration to afforded 8.5 g of 2-(3,4-dimethoxyphenyl) acetamide as a dark brown gummy oil.

Step-02: Preparation of N-(1-(1*H*-benzo[d][1,2,3]triazol-1-yl)-2,2-dimethylpropyl)-2-(3,4-dimethoxyphenyl) acetamide (1): 2 g of step-1 compound *i.e.*, 2-(3,4-dimethoxyphenyl)acetamide (1 eq) was dissolved in 40 mL of toluene (20 times w/v) to this clear solution added 1.85 g trimethyl acetaldehyde (2 eq), 1.21 g of 1*H*-benzotriazole (1 eq) and 0.097 g of *p*-toluenesulfonic acid at ambient temperatures. The reaction mixture was heated to reflux and collected water by arranging Dean-Stark apparatus (about 2-4 h). The reaction mixture was stirred at 60-65 °C till the reaction completion (about 10-12 h) and reaction was monitored by TLC (mobile phase for TLC 100 % ethyl acetate) after completion of the reaction. The reaction was cooled to room temperature (*ca.* 30 °C) and solvent was removed by evaporation. The residue obtained was dissolved 50 mL of ether (25 times w/v) and the precipitated solid was collected by filtration to afforded 1.3 g of N-(1-(1*H*-benzo[d][1,2,3]triazol-1-yl)-2,2-dimethylpropyl)-2-(3,4-dimethoxyphenyl)acetamide (**1**) as a solid and structure was confirmed by following analysis. ^{1}H-NMR ($CDCl_3$) δ: 1.06 (s, 9H, -$(CH_3)_3$), 3.44 (s, 2H, -CH_2-), 3.73 (s, 6H, -OCH_3), 5.77 (s, 1H, -CH-), 6.4-6.6 (m, 3H, Ar-H), 7.4-7.8 (m, 4H, Ar-H), 8.0 (s, 1H, NH). IR (KBr, ν_{max}, cm^{-1}):

2822 (-OCH_3), 2891 (C-H), 1600, 1575, 1452 (C-C), 3454 (N-H), 1689 (C=O), 1592 (N=N). EL-MS m/z (M^+): 383 (calculated for $C_{21}H_{26}N_4O_3$: 382.46). Elemental analysis for $C_{21}H_{26}N_4O_3$: C-65.95, H-6.85, N-14.65; found: C-65.91, H-6.89, N-14.68.

Synthesis of other compounds from 2 to 5

N-(1-(1*H*-Benzo[d][1,2,3]triazol-1-yl)-2,2-dimethylpropyl)-2-(3,4-diethoxyphenyl)acetamide (2): To get title compound 2-(3,4-diethoxyphenyl)acetic acid was used instead of 2-(3,4-dimethoxyphenyl)acetic acid in the above general synthesis 1.2 g of N-(1-(1*H*-benzo[d][1,2,3]triazol-1-yl)-2,2-dimethylpropyl)-2-(3,4-diethoxyphenyl)acetamide (**2**) was obtained as solid and structure was confirmed by following analysis. ^{1}H NMR ($CDCl_3$) δ: 1.06 (s, 9H, -$(CH_3)_3$), 1.33 (t, 6H, -CH_3), 3.44 (s, 2H, -CH_2-), 3.98 (q, 4H, -OCH_2-), 5.77 (s, 1H, -CH-), 6.4-6.6 (m, 3H, Ar-H), 7.4-7.8 (m, 4H, Ar-H), 8.0 (s, 1H, NH). IR (KBr, ν_{max}, cm^{-1}): 2819 (-OCH_2-), 2985 (C-H), 1600, 1575, 1452 (C-C), 3454 (N-H), 1689 (C=O), 1592 (N=N). EL-MS m/z (M^+): 411.2 (calculated for $C_{23}H_{30}N_4O_3$: 410.51). Elem. analysis for $C_{23}H_{30}N_4O_3$: C-67.29, H-7.37, N-13.65; found: C-67.31, H-7.39, N-13.58.



Compound	R	R^1	R^2	Chemical name
1	H	OCH_3	OCH_3	N-(1-(1*H*-Benzo[d][1,2,3]triazol-1-yl)-2,2-dimethylpropyl) -2-(3,4-dimethoxyphenyl) acetamide
2	H	OC_2H_5	OC_2H_5	N-(1-(1*H*-Benzo[d][1,2,3]triazol-1-yl)-2,2-dimethylpropyl)- 2-(3,4-diethoxyphenyl)acetamide
3	H	OH	OH	N-(1-(1*H*-Benzo[d][1,2,3]triazol-1-yl)-2,2-dimethylpropyl)- 2-(3,4-dihydroxyphenyl)acetamide
4	H	H	OCH_3	N-(1-(1*H*-Benzo[d][1,2,3]triazol-1-yl)-2,2-dimethylpropyl) -2-(3-methoxyphenyl)acetamide
5	H	Br	H	N-(1-(1*H*-Benzo[d][1,2,3]triazol-1-yl)-2,2-dimethylpropyl) -2-(4-bromophenyl)acetamide

Scheme-I

N-(1-(1*H*-Benzo[d][1,2,3]triazol-1-yl)-2,2-dimethylpropyl)-2-(3,4-dihydroxyphenyl)acetamide (3): To get title compound 2-(3,4-dihydroxyphenyl) acetic acid was used instead of 2-(3,4-dimethoxyphenyl)acetic acid in the above general synthesis 1.2 g of N-(1-(1*H*-benzo[d][1,2,3]triazol-1-yl)-2,2-dimethylpropyl)-2-(3,4-dihydroxyphenyl)acetamide (**3**) was obtained as solid and structure was confirmed by following analysis. ^{1}H NMR ($CDCl_3$) δ: 1.06 (s, 9H, -$(CH_3)_3$), 3.44 (s, 2H, -CH_2-), 5.0 (s, 2H, -OH), 5.77 (s, 1H, -CH-), 6.4-6.6 (m, 3H, Ar-H), 7.4-7.8 (m, 4H, Ar-H), 8.0 (s, 1H, NH). IR (KBr, ν_{max}, cm^{-1}): 3580 (O-H), 2985 (C-H), 1600, 1575, 1452 (C-C), 3454

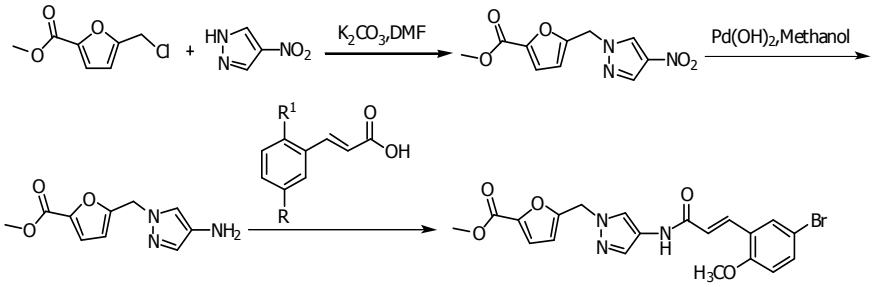
(N-H), 1689 (C=O), 1592 (N=N). EL-MS m/z (M^+): 355.6 (calcd. for $C_{19}H_{22}N_4O_3$: 354.4). Elemental analysis for $C_{19}H_{22}N_4O_3$: C-64.39, H-6.26, N-15.81; found: C-64.45, H-6.30, N-15.72.

N-(1-(1*H*-Benzo[d][1,2,3]triazol-1-yl)-2,2-dimethylpropyl)-2-(3-methoxyphenyl)acetamide (4): To get title compound 2-(3-methoxyphenyl)acetic acid was used instead of 2-(3,4-dimethoxyphenyl)acetic acid in the above general synthesis 1.25 g of N-(1-(1*H*-benzo[d][1,2,3]triazol-1-yl)-2,2-dimethylpropyl)-2-(3-methoxyphenyl)acetamide (**4**) was obtained as solid and structure was confirmed by following analysis. ^{1}H NMR ($CDCl_3$) δ: 1.06 (s, 9H, -$(CH_3)_3$), 3.44 (s, 2H, -CH_2), 3.73 (s, 3H, -OCH_3), 5.77 (s, 1H, -CH-), 6.4-6.6 (m, 3H, Ar-H), 7.4-7.8 (m, 4H, Ar-H), 8.0 (s, 1H, NH). IR (KBr, ν_{max} cm^{-1}): 2835 (OCH_3), 2985 (C-H), 1600, 1575, 1452 (C-C), 3454 (N-H), 1689 (C=O), 1592 (N=N). EL-MS m/z (M^+): 353.5 (calcd. for $C_{20}H_{24}N_4O_2$: 352.43). Elemental analysis for $C_{20}H_{24}N_4O_2$: C-68.16, H-6.86, N-15.90; found: C-68.25, H-6.80, N-15.92.

N-(1-(1*H*-Benzo[d][1,2,3]triazol-1-yl)-2,2-dimethylpropyl)-2-(4-bromophenyl)-acetamide (5): To get title compound 2-(4-bromophenyl)acetic acid was used instead of 2-(3,4-dimethoxyphenyl)acetic acid in the above general synthesis 1.1 g of N-(1-(1*H*-benzo[d][1,2,3]triazol-1-yl)-2,2-dimethylpropyl)-2-(4-bromophenyl)-acetamide (**5**) was obtained as solid and structure was confirmed by following analysis. ^{1}H NMR ($CDCl_3$) δ: 1.06 (s, 9H, -$(CH_3)_3$), 3.44 (s, 2H, -CH_2-), 5.77 (2, 1H, -CH-), 6.4-6.6 (m, 3H, Ar-H), 7.4-7.8 (m, 4H, Ar-H), 8.0 (s, 1H, NH); IR (KBr, ν_{max}, cm^{-1}): 2835 (OCH_3), 2985 (C-H), 1600, 1575, 1452 (C-C), 3454 (N-H), 1689 (C=O), 1592 (N=N). EL-MS m/z (M^+): 402.5 (calcd. for $C_{19}H_{21}BrN_4O$: 401.3). Elemental analysis for $C_{19}H_{21}BrN_4O$: C-56.87, H-5.27, Br-19.91, N-13.96; found: C-56.80, H-5.29, Br-19.99, N-13.89.

Similarly methyl 5-((4-(2,5-disubstituted phenyl)furan-2-carboxylate derivatives were prepared (**Scheme-II**).

Cardioprotective activity: All the compounds were tested for cardioprotective activity by ischemia reperfusion method, administering compounds in a dose of 20 mg/kg body weight of the animals. Albino Wilstar rats of either sex weighing 175-225 g were used (Supplied by Mahavir Enterprises, Hyderabad, India). Rats were divided randomly into 5 groups each. All the rats were anaesthetized with thiopental sodium (40 mg/kg body weight of the animal, intraperitoneally) and were ventilated with room air by using the Techno positive pressure artificial respirator. Ventilatory parameters were adjusted to maintain satisfactory oxygenation. The chest was opened through fourth intercostal space at left side and heart was exposed by removing pericardium and the left coronary artery was located silk thread was passed below the left coronary artery and was occluded for 0.5 h to allow reperfusion of the heart for 4 h. Femoral vein was annulated to administer 80 % DMSO and drug compounds were administered intravenously through femoral vein at 28th min of occlusion.



Compound	R^1	R^2	Chemical name
6	Br	OCH_3	Methyl 5-((4-(-3-(5-bromo-2-methoxyphenyl)acrylamido)-1*H*-pyrazol-1-yl)methyl) furan-2-carboxylate
7	F	OCH_3	Methyl 5-((4-(-3-(5-Fluoro-2-methoxyphenyl)acrylamido)-1*H*-pyrazol-1-yl)methyl)furan-2-carboxylate
8	Br	OC_2H_5	Methyl 5-((4-(-3-(5-Bromo-2-ethoxyphenyl)acrylamido)-1*H*-pyrazol-1-yl)methyl)furan-2-carboxylate
9	Br	OH	Methyl 5-((4-(-3-(5-Bromo-2-hydroxyphenyl)acrylamido)-1*H*-pyrazol-1-yl)methyl)furan-2-carboxylate
10	Cl	NO_2	Methyl 5-((4-(-3-(5-Chloro-2-nitrophenyl)acrylamido)-1*H*-pyrazol-1-yl)methyl)furan-2-carboxylate

Scheme-II

Quantification of infract size: At the end of 4 h of reperfusion, animals were sacrificed. Heart was excised from thorax rapidly and the greater vessels were removed. Myocardial infract size was expressed quantitatively in terms of per cent left ventricle necrosis (PLVN), tissue malondialdehyde (MDA) concentrations and were estimated. Lipid peroxidation is group often the first parameter to prove the involvement of free radicals in cell damage. Free radicals undergo reaction with polyunsaturated fatty acids in the phospolipids of cellular membranes to yield lipid hydroperoxides (LOOH). The LOOH and conjugated dienes that are formed can decompose to form numerous other products including alkanals, alkenals hydroxyl alkenals, MDA and volatile hydrocarbons[12]. On such approach is the detection of MDA concentration, MDA levels increase in presence of increased free radical activity[13]. The PLVN and tissue MDA results are expressed in Figs. 1 and 2, respectively.

Data analysis: All the values presented are mean ± SEM. Differences in PLVN and tissue MDA levels were determined by one way ANOVA followed by dunnet's test. A Level of $p < 0.05$ was accepted as statistically significant. Statistical analysis was performed using Sigma Plot Software (Version 10).

RESULTS AND DISCUSSION

The per cent left ventricular necrosis and MDA concentrations in all groups at the end of 4 h of reperfusion is shows in Figs. 1 and 2, respectively. In control untreated animals PLVN and MDA were found to be 50.73 ± 0.76 and 108.86 ± 0.86 %, respectively. The results obtained indicate that all the synthesized compounds

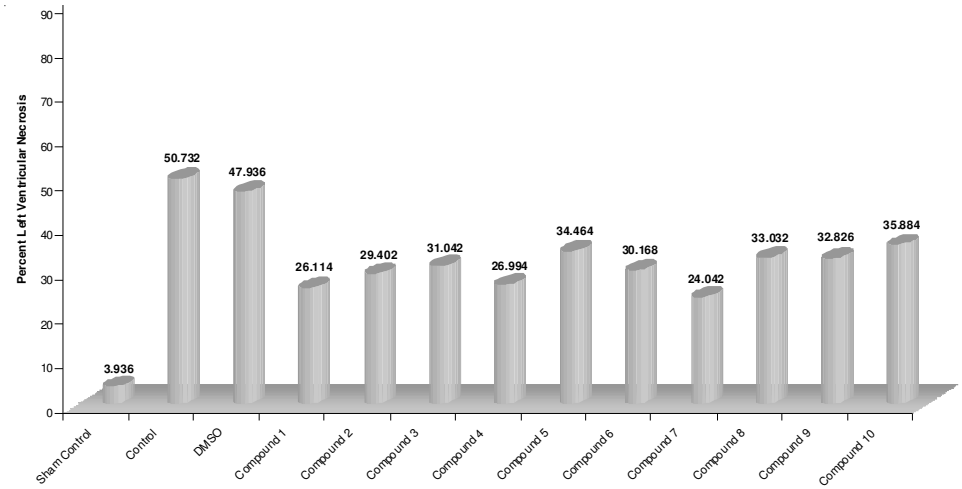


Fig. 1. Per cent left ventricular necrosis for sham control, control, DMSO ad compounds (**1-10**)

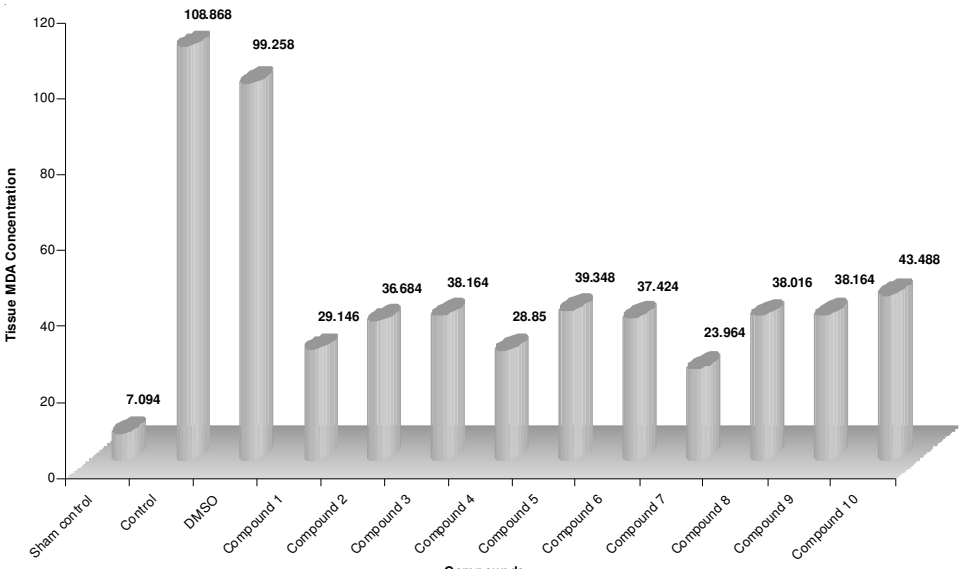


Fig. 2. Tissue MDA concentration for sham control, control, DMSO and compounds (**1-10**)

significantly lowered PLVN and MDA concentrations in compared to control group treated with dimethyl sulfoxide, when administered in a dose of 20 mg/kg body weight of the animal. It was also observed that compound **7** shows higher activity among all the compounds. Alkoxy substituted dimethoxy, diethoxy compounds show more activity while halogen substituted compounds with less activity and nitro substituted compounds shows moderate activity.

The search for new therapeutic agents in recent years by understanding of the structure of enzymes and other biomolecules associated with target disease. One important class of such enzymes is protein kinase. There are number of the kinases and pathways through which extra cellular and other stimuli cause a variety of cellular response occur inside the cell. Many disorders are associated with abnormal cellular response triggered by protein kinase mediated events. These diseases include autoimmune diseases, inflammatory diseases, neurological and neurodegenerative diseases, cancer, cardiovascular diseases. Substituted pyrazole compounds are reported as protein kinase inhibitors. Potassium channels play an important role in regulating cell membrane exicisatability. When potassium channels opens changes in electrical potential across the cell membrane occurs and resulting a more polarized state. A number of diseases and conditions may be treated with therapeutic agents that open potassium channels. Such diseases and conditions includes asthma, epilepsy and bladder over activity. Potassium channel openers are useful in the treatment of myocardial injury and heart diseases. Nitrogen containing heterocyclics have diverse therapeutic applications. In the present study we have evaluated the cardio protective activities of some novel substituted benzotriazole and pyrazole derivatives in the experimental ischemia reperfusion induced myocardial infraction in rats. They have offered good cardio protective activity by significantly reducing the infract size and tissue MDA concentrations when compared to control group treated with dimethyl sulfoxide. Many mechanisms are involved in ischemia reperfusion injury. Role of reactive oxygen species (ROS)[14] role of cardiac rennin-angiotensin system (RAS)[15] role of sympathetic nervous system[16] role of Na^+/H^+ exchange[13], role of neutrophils[17], role of inflammatory mediators[18] role of platelets[16] role of calcium[19]. Protein kinase (PKC)[20], role of caspases[21], role of P-38 mitogen-activated protein kniase[22]. The cardio protective activity of synthesized title compounds is proved by their capacity to reduce PLVN and tissue MDA concentration. As 5-substituted-1-(2-hydroxy benzoyl)-benzotriazoles are activators of potassium channels and as with full vasoreflexing efficacy the observed cardioprotective activity of synthesized novel benzotriazoles compounds may be because of their antiinflammatory, antiplatelet activity or may be due to their potassium channel activating and the cardioprotective activity of pyrazole compounds may be because of their protein kinase inhibitory activity.

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