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SYNTHESIS AND BIOLOGICAL SCREENING OF SOME PYRIDAZINE DERIVATIVES

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Abstract: As a part of research project, the knowledge gained by various literatures has suggested that substituted pyridazine which possess good pharmacological activity with lower toxicities. In recent years 6-aryl pyridazine shows promising Antihypertensive activity. So substituting or adding a new moiety to the parent lead compound thus by making gradual changes in the structure of compound resulting gradual change in physicochemical properties and biological activities of drug. Firstly phenoxyacetylchloride was prepared from Phenol and Chloroacetic acid and thionyl chloride. Then it reacted with different aromatic and amines and then after reaction with maleic anhydride in presence of $AlCl_3$. Then after reaction with semicarbazide in presence of ethanol gives different pyridazine derivatives. All the synthesized compounds were characterized by UV, IR, Mass and some of by ^{1}H-NMR spectroscopy & report of them supports the structures of compounds. Compounds 51b, 51c, were found to be having significant antihypertensive effect as compared to control and standard. Other compounds like 51a, 51f, 51e and 51d were found to be lower active as compared to standard and other derivatives. All the synthesized final compounds were screened for antihypertensive activity by *in vitro* Method against standard reference drug Hydralazine. Synthesized compound having pyrrolidine ring having gives moderate antihypertensive activity. When pyrrolidine ring was replaced by heterocyclic rings like piperidine, N-methyl piperazine, morpholine, Phenylhydrazine and aniline the antihypertensive activity was altered and has exhibited less significant effect.

Keywords: Antihypertensive, Pyridazine, Hydralazine, Aromatic Amines,

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INTRODUCTION

Hypertension is a disorder of the cardiovascular system characterized by elevated arterial blood pressure. The blood pressure in the arteries is dependent on the energy of cardiac contractions, elasticity, and contractile state of arterial walls, as well as on the volume and viscosity of the blood. It fluctuates with every heartbeat. Blood Pressure involves 2 measurements, *systolic* and *diastolic.* Normal Blood Pressure is 120/80 mm Hg. Maximal pressure occurs near the end of the stroke output and is termed as *systolic*. Minimal pressure occurs late in ventricular diastole and is termed as *diastolic*. High blood pressure is anything above 140/90 mm Hg. Hypertension is opposite of Hypotension. Hypertension is classified as either **Primary** (Essential) hypertension (90-95%) which means high blood pressure with no obvious medical cause or **Secondary** hypertension (5-10%) which caused by other condition those effects kidneys, arteries, heart or endocrine system. Systolic blood pressure for Individual is at or less than 120 mmHg and Diastolic blood pressure for individual is at or less than 80 mmHg are considered "*normotensive"*. The term *'prehypertensive'* is used for individuals with a systolic pressure under 140, but above 120 mmHg and diastolic pressure under 90, but above 80 mmHg. Patients with 140–159 mmHg systolic and over 90 mmHg diastolic have "***phase-I*** *hypertension"*, while patients with systolic pressure over 160mmHg and diastolic pressure over 100 mmHg have *"**phase-II** hypertension".* Hypertension is usually systemic, but it may be limited to certain organs. The term *'pulmonary hypertension'* is used to describe a selective elevation of pressure in pulmonary arteries. Portal hypertension, a persistent elevation of pressure in portal veins, is usually a secondary hypertension that often occurs as a consequence of liver cirrhosis.[1-4] Hypertension is a common disorder that, if not effectively treated, increases the risk of coronary thrombosis, strokes and renal failure. In market different pyridazine analogous having antihypertensive activity (eg.Hydralazine, Dihydralazine, cadralazine) are available. But most of having a side effect like, headaches, weakness, flushing, and nausea. These drugs also cause fluid retention and tachycardia (fast heart rate), so doctors usually prescribe them in combination with a cardioselective beta blocker. In order to overcome this situation, it is necessary to continue the search for new antihypertensive agents. From the literature review we find that pyridazine derivatives was found to be having activity like anti-hypertensive, anti-proliferative, anti-inflammatory activity, anti-fungal activity.[5-12] Therefore it was aimed to synthesize the novel series of pyridazine and its derivatives and to evaluate the biological activities of newly synthesized compounds.

Method and Materials

Thin layer chromatography was performed on microscopic plates (2×7.5cm) coated with silica-gel-60 and spots were visualized under UV light and by exposure to iodine vapour. UV spectra were recorded in UV-1700 Shimadzu spectrophotometer. IR spectra of all compounds were

recorded on FT-IR 8400S Shimadzu Spectrophotometer using KBr and Bruker alpha ATR. Mass spectra were obtained on request from Synzeal laboratory using LCMS Shimadzu instrument. The 1H-NMR was recorded on Bruker advanced–II NMR-400MHz instruments using $CDCl_3$ as solvent and Tetra methyl silane (TMS) as internal standard, chemical shifts were expressed as δ values (ppm).

Procedure for synthesis of Phenoxy acetic acid[31](2)

To a mixture of (4.7 g, 0.05 mole) chloroacetic acid, and (4.8 g, 0.05 mole) phenol, 25 ml solution of 4.8 mole/L aqueous sodium hydroxide was added slowly with constant stirring. The mixture was stirred for 2 h till solution turn greenish-yellow. Then the solvent was evaporated till sodium salt precipitated out. The salt was dissolved in water and acidified with conc. HCl. The precipitate was filtered off and recrystallized from ethanol.

Procedure for synthesis of Phenoxy Acetyl Chloride[31](3)

A mixture of (0.5 g, 0.003 mole) phenoxy acetic acid and (0.22 ml, 0.003 mole) thionyl chloride and DCM was refluxed for 3-4 hrs. The excess of thionyl chloride was distilled out. After cooling in ice-bath a viscous liquid was obtained.

General procedure for synthesis of amide derivatives (4a-4f)

0.1 mole Phenoxy acetyl chloride was reacted with 0.1 mole of aromatic amines drop wise. Though the reaction is explosive, care should be taken while performing the reaction and also it was carried out in ice bath. The product was purified by washing with diethyl ether. The product was isolated as solid and reaction was monitored by thin layer chromatography.

General procedure for synthesis of substituted keto acid (5a-5f)

A mixture of amide derivatives (49a) (8 mmol) and maleic anhydride (1.25 g, 12 mmol) was added to a stirred solution of aluminium chloride (5 g) in ether (10 ml). The reaction mixture was stirred manually under the anhydrous conditions for 20 min and then allowed to stand for 48 h at room temperature. Remove ether and the resultant solution was filtered and then cooled in ice for precipitation. The solid product obtained was filtered and washed thoroughly with distilled water. The solid residue was dissolved in 10% aqueous sodium bicarbonate solution and filtered off insoluble part. Acidification with concentrated hydrochloric acid gave precipitate, which was filtered, washed with water and recrystallized from methanol.

General procedure for synthesis of 3-{4-[2-(substituted)-2-oxoethoxy]phenyl}-6-oxopyridazine-1(6*H*)-carbohydrazide (6a-6f)

Carbohydrazide (1 mmol) was added to a stirred and refluxing solution of substituted keto acids (50a) (1 mmol) in aldehyde free ethanol (50 ml). The reaction mixture was further refluxed for 8 h with continuous stirring. The completion of the reaction was monitored by TLC. The solvent was removed under reduced pressure to obtain a solid residue. Ice-cold water was added to it and the precipitate obtained was filtered off, washed with ice-cold water, dried and recrystallized.

Biological screening

The animals will be kept under standard laboratory conditions in 12 h light/dark cycle at 25±2˚C. Animals will be provided with pellet diet and water *ad libitum*. They will be marked for easy identification. There will be three animals in each group. The animals are sacrificed by inhalation of CO_2. The descending thoracic aorta was rapidly dissected and placed in a physiological saline solution. After excess of fat and connective tissue will be removed, the aorta will be cut into rings (4–5 mm in length), mounted under the basal tension of 2 g in 5 ml organ baths containing PSS and attached to force-displacement transducers to measure isometric contractile force. The tissue bath was maintained at 37˚C and bubbled with O_2/CO_2 (95:5) gas mixture. Each preparation was allowed to equilibrate for at least 90 min prior to initiation of experimental procedures and during this period the incubation media will be changed every 20 min. After equilibration aortic rings will be contracted by single concentration of phenylephrine (10^{-6}M). When the contractions will be stable, compounds will be added in progressively increasing cumulative concentrations at 30 min intervals. Only one compound was tested in each ring. All compounds will be initially dissolved in dimethyl sulfoxide (DMSO) to prepare a 10^{-2}M stock solution. Further solutions will be made in PSS. The response of the aortic rings to all compounds was expressed as a percentage of the initial contraction to 10^{-6}M phenylephrine.[12,16,17] The antihypertensive activity displayed by various compounds (6a-6f) is shown in Table-3

Result and Discussion

The structure of (5a-5f) were confirmed by IR, MASS, ^{1}H-NMR shown as below;

Compound 5a- IR: 2801 (-COOH), 1732 (C=O), 1161 (ether); Mass (m/z): 330.1 (M-2), 331.1 (M-1), 332.1 (M); Compound 5b- IR: 1715 (C-O), 1019 (ether), 2812 (-COOH),

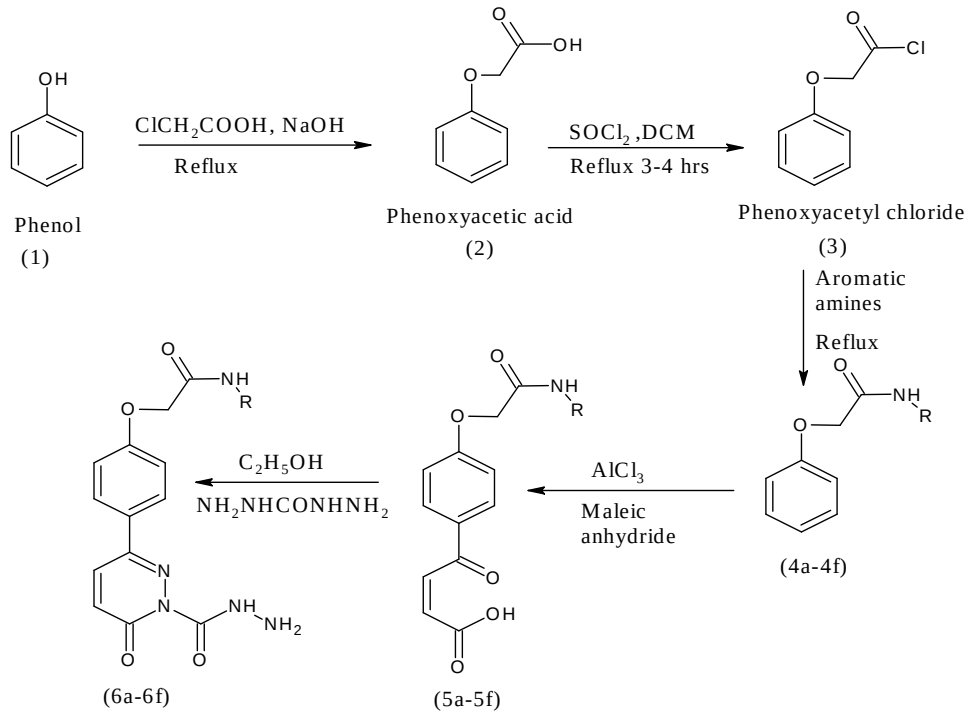
3109(C-H stretch); Mass (m/z): 303.1 (M-1); Compound 5c- IR: 1219 (ether), 1712 (C=O), 3205 (-COOH), 3385 (C-H stretch); Mass (m/z): 318.2 (M-1); Compound 5d- IR: 1214 (ether), 1723 (C=O), 3052 (-COOH), 3273 (C-H stretch); Mass (m/z): 316.2 (M-1); Compound 5e- IR: 1262 (ether), 1730 (C=O), 3200 (-COOH), 3297 (N-H); Mass (m/z): 339.1 (M-1); Compound 5e- 1091

(ether), 3097 (-COOH), 3257 (N-H); Mass (m/z): 324.1(M-1); ^{1}H-NMR(δ, ppm): 9.96 (s, 1H, -NH-), 8.22-6.61 (m, 11H, Ar-H) 4.66 (s, 2H, -CH_2-).

Physical and spectral characteristics of synthesized final compounds (6a-6f) are presented in Table-1 and Table-2 respectively.

All the synthesized compounds ware characterized by using IR, Mass and some by NMR spectroscopy. From the above activity table it is evident that the entire synthesized compounds give lower antihypertensive activity than standard drug. Compound 51b, 51c, were found to be having more activity compared to standard and other synthesized derivatives. Compound 51a, 51f, 51e, 51d were found to be having lower activity than other derivatives. All synthesized compound were screened for antihypertensive activity by in vitro method.

Conclusion: All the synthesized final compounds were screened for antihypertensive activity by *in vitro* method against standard reference drug Hydralazine. Synthesized compound having pyrrolidine ring having gives moderate antihypertensive activity. When pyrrolidine ring was replaced by heterocyclic rings like piperidine, N-methyl piperazine, morpholine, the antihypertensive activity was altered and has exhibited less significant effect. Evaluation of antihypertensive activity of compound pyrrolidine substituted 6-aryl pyridazine gives moderate biological activity. Compound 51d having less biological activity.



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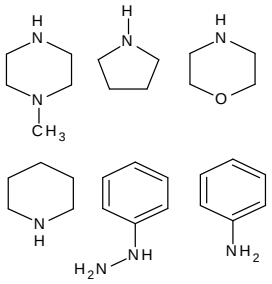

Table -1 Physical Characteristics of Synthesized Compounds (6a-6f)

Compound Code	Molecular Formula	Molecular Weight (g/mol)	Yield (%w/w)	Melting Point (oC)
6a	$C_{18}H_{22}N_6O_4$	386.40	42.3	160-162
6b	$C_{17}H_{19}N_5O_5$	357.36	39.6	207-209
6c	$C_{17}H_{19}N_5O_4$	373.36	32.6	171-173
6d	$C_{18}H_{21}N_5O_4$	371.39	38.2	165-167
6e	$C_{19}H_{18}N_6O_4$	394.38	42.5	217-219
6f	$C_{19}H_{17}N_5O_4$	379.36	34.6	197-199

Table -2 Spectral Data of Synthesized Compounds (6a-6f)

Comp. Code	UV λmax (nm)	IR (υ, cm-1)	Mass (m/z)	1H NMR (δ, ppm)
51a	237nm	3200 (NH_2), 1716 (C=O), 1249 (ether), 3092 (C-H stretch)	385.3 (M-1), 387.3 (M+1)	_
51b	236nm	3239 (-NH_2), 1748 (C=O), 1212 (ether), 3025 (C-H stretch)	358.1 (M+1)	_
51c	238nm	3113 (-NH_2), 1730 (C=O), 1245 (ether)	374.2 (M+1)	_
51d	237nm	3341 (-NH_2), 1731 (C=O), 1249 (ether), 3233 (C-H stretch)	372.4 (M+1)	_
51e	242nm	3297 (-NH_2), 1703 (C=O), 1169 (ether) 3013 (C-H stretch)	395.2 (M+1)	_

51f	257nm	3345 (-NH_2), 1710 (C=O), 1241 (ether),	380.1 (M+1)	9.96 (s, 1H, -NH-), 8.63 (s, 1H, -NH-) 7.78-6.74 (m, 11H, Ar-H), 4.66 (s, 2H, -CH_2-), 4.35 (s, 2H, -NH_2)

Table-4 Screening of antihypertensive activity of Hydralazine.

Concentration (µg/ml)	Dose (mL)	Control (H=cm)	Test (h=cm)	% Inhibition	IC_{50}
Hydralazine	0.1	2.3	1.9	8.16	0.46
	0.2	2.8	2.1	14.2	
	0.4	3.4	2.4	20.4	
	0.6	3.9	2.5	28.5	
	0.8	4.2	2.5	34.7	
	1	4.9	2.7	44.9	

Table-3 Screening of antihypertensive activity of Synthesized Compounds (6a-6f)

Compound Code	Dose (mL)	Control (H) (cm)	Test (h) (cm)	% Inhibition	IC_{50} (µg/ml)
51a	0.1	1.1	1	4.76	1.22
	0.6	1.5	1.3	9.52	
	1	2.1	1.7	19.04	
51b	0.1	1.5	1.1	17.39	0.72
	0.6	1.9	1.4	26.08	
	1	2.3	1.5	34.78	
51c	0.1	1.4	1.1	13.63	1.04
	0.6	1.7	1.3	18.18	
	1	2.2	1.6	27.27	
51d	0.1	1.2	1	10.25	1.45

	0.6	1.7	1.4	15.32	
	1	2	1.6	20.45	
51e	0.1	1.2	1	9.09	1.68
	0.6	1.5	1.2	13.63	
	1	2.2	1.8	18.09	
51f	0.1	1.4	1.2	8.07	1.86
	0.6	1.8	1.5	12.18	
	1	2.5	2.1	16.42	

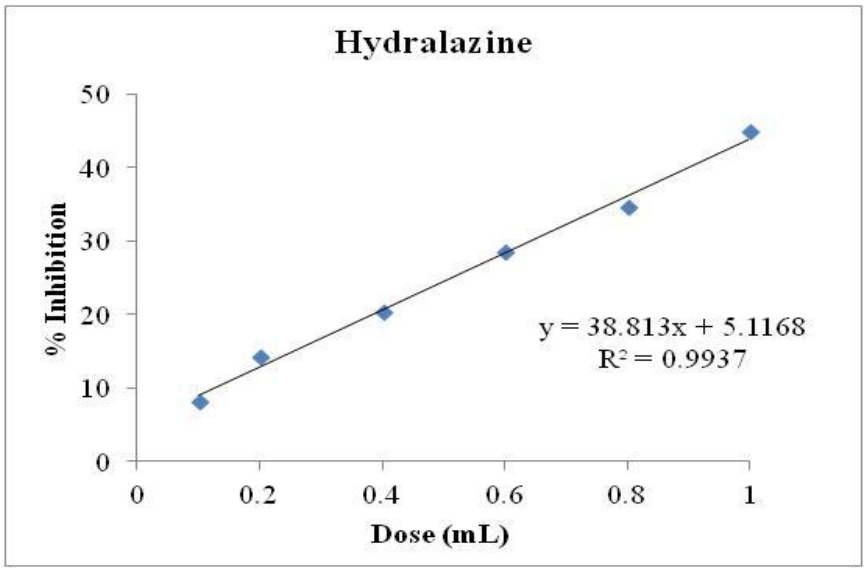

Figure 1: % inhibition vs. dose curve of Hydralazine

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