SYNTHESIS AND EVALUATION OF NOVEL PHENACYL BASED CARBOXAMIDE DERIVATIVES OF PIPERIDINE AS ANTIHYPERTENSIVE AGENTS

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Background: The research in the field of piperidine has generally been related to the synthesis of useful medicinal drugs. In view of the pharmacological and medicinal importance of piperidine derivatives in different disciplines of medicines the present study has been carried out. **Methods:** A series of newly synthesized N-substituted phenacyl piperidine derivatives (II-VII and XIII-XIV) has been evaluated for hypotensive activity in normotensive anesthetized rats at the doses of 0.5µg/kg taking acetylcholine and noradrenaline (1µg/kg) as control. Mean arterial blood pressure and heart rate were compared to its respective control values obtained immediately before the administration of test compounds and expressed as percent change. **Results:** The compounds II, III and XIV showed mild hypertensive activity while compound V, VII and XIII were found inactive at that dose level. However, compound IV showed more hypotensive effect than the starting molecule (I). None of these derivatives affected the heart rate at the same dose. It was also revealed that the carboxamide group has no considerable effects on arterial blood pressure and playing no important role in the increase or decrease of blood pressure.

Key words: phenacyl-carboxamide derivatives, MABP, normotensive, anesthetized rats, heart rate.

INTRODUCTION

The physiological activity of many substituted piperidines prompted the scientists to design simple methods for the synthesis of piperidine carboxamide derivatives. During the studies of correlating the structure and cardiovascular activity in the past several decades, various derivatives of piperidine-carboxamide compounds (hexahydro-nicotinamide and -isonicotinamide) had been reported by various workers.[1-3] They found to possess spasmolytic[4] and antihypertensive activity.[5-6]

In searching of new hypotensive agents, substituted hexahydronicotinamides were prepared by Swaim *et al*.[7] They caused a decrease in blood pressure after intravenous injections into dogs anaesthetized with α-chlorates while 1-(3-indolylmethyl N,N-diethyl) hexahydronicotinamide was found to lower blood pressure, decrease the heart rate and block the pressure rise[8] resulting from bilateral carotid occlusion at dose level of 2-16 mg/kg. Another group of workers reported a piperidine derivative prepared from 1-benzoyl-3-propyl-4-piperidinone with 4 FC_6H_4Br, was a rennin inhibitor with IC_{50} of 0.317 µM for the treatment of high blood pressure, heart and kidney insufficiency. In 2001, Agarwal, Vijay[9] and coworkers studied structure activity relationship on antihypertensive activity of a series of 4-(diarylmethyl)-N-substituted piperidine. The data were found statistically significant. Saify *et al*.[10] synthesized several new derivatives of 4-hydroxy-4-phenyl piperidine among them three potent hypotensive compounds have been evaluated.

The cardiac activity of piperidine derivatives was found to be due to their Ca^{++} channel blocking effects.[11-13] Compounds such as 2- and 6-methyl substituted (3, 4-dihydroxy phenyl)-3-piperidinol derivatives synthesized for their α-adrenergic activity offering useful information about the steric requirement for the direct activation of α_1 and α_2 adrenergic receptors.[14-15] Aryl and alkyl substituted derivatives of piperidine and their salts were found useful as acetylcholine esterase inhibitors and showed IC_{50} of 0.7 µM against specific uptake of ^{45}Ca.[16]

Watanabe, Nobuhisa *et al*.[17] in 2000 have synthesized different derivatives of piperidine with 4-(3-chloro-4-methoxybenzyl) aminophthalazines substituted at 1 and 6 positions. These compounds were evaluated as phosphodiesterase 5 inhibitors. One of the compounds exhibited most potent vasorelaxant action and reduced mean arterial pressure by 29.9±3.1% when administered (i.v.) at 30 µg/kg to chronically hypoxic rats while an apparent oral bioavailability of about 19.5% was observed in the same dose when administered orally.

In search of potent antihypertensive agents number of piperidine derivatives have been investigated for their blood pressure lowering activity and were found active.[18-19] Considering the previous findings,[20] it has been decided to test the derivatives of Hexahydroisonicotinamide (isonipecotamide) for their effects on mean arterial blood pressure (MABP)

and heart rate to have more potent hyper or hypotensive agents.

MATERIAL AND METHODS

The chemicals and solvents used were purchased from Sigma Co., (Aldrich, London) and Merk. The melting point of the compounds recorded in glass capillary tubes on Galen kamp melting point apparatuses and were uncorrected.

^{1}H nmr spectra were run in CD Cl3/d_6 DMSO on Broker AM 300 and Broker AM 400 NMR spectrometers operating at 300 and 400 MHZ. The chemicals shift were recorded in PPM (d) and coupling constant (J) are in HZ. Mass spectra were measured on Finnegan MAT 112 11/34 computer system.

The compounds were tested for their effects on mean arterial blood pressure and heart rate by the method as described earlier by McLeod[21] and Thomas *et al*.[22] with slight modifications as described by S. Khatoon.[23] Normotensive anaesthetized male wistar rats (weighing 220gm) were used for this study. Animals were anaesthetized with an intraperitoneal injection of sodium pentothal (70-90 mg/kg body weight). The arterial blood pressure was recorded from the right carotid artery via the arterial cannula connected to a pressure transducer coupled with a Grass 7D model polygraph to monitor the blood pressure of animals. The left juglar vein was cannulated with similar tubing to facilitate the I.V. injection of the drugs and test compounds. The rats were injected with heparin (1000 µg/kg body weight) to prevent blood clotting. The body temperature of animals was monitored via rectal temperature probe of differential thermometer (Exacon, MC 8940) and maintained at 37°C by using an overhead lamp.[24] Animals were allowed to equilibrate for at least 20 minutes prior to the administration of acetylcholine and noradrenaline (1 µm/kg) taken as controls. All the test compounds were administered as a bolus injection of single dose at a volume of 0.5 ml/kg, followed by a saline flush of 0.2 ml.[25] Mean blood pressure was calculated as: Diastolic blood pressure plus one-third of pulse pressure (Blood pressure = Diastolic blood pressure + 1/3 pulse pressure).

To obtain the pulse pressure, diastolic blood pressure was subtracted from the systolic blood pressure. The heart rate was calculated manually by counting the excursions of the blood pressure tracings, after running the tracing at high speed.[26] Blood pressure and heart rate were compared to its respective control values obtained immediately before the administration of test compounds and expressed as percent change.

General method for synthesis of compounds (II-V & XIII-XIV)

Isonipecotamide (1) and corresponding substituted phenacyl halides (2) were dissolved in acetone in equimolar quantities separately in conical flasks heating on water bath and then mixed together in a round bottom flask. The reaction mixtures were stirred on magnetic stirrer for about 4 to 5 hours. Precipitates of products appeared on mixing the reactants and in some cases after refluxing on water bath and setting aside at room temperature. The completion of reactions was monitored by TLC using $CHCl_3$-MeOH as solvent system. The resulting products were filtered and washed with warm acetone to remove the unreacted starting materials. The products thus obtained were recrystallized from methanol at least three times to ensure purity and to improve color and shape of the crystals.

4-Carbamoyl-[2-(3,4-dihydroxyphenyl)-2-oxoethyl]-piperidinium chloride (II)

Gray crystalline powder obtained from methanol (40%), **mp** 222 – 224°C. **IR** m**max** 3384 ($CONH_2$), 3347 (OH), 2973 (CH_2), 1676 (C=O), 1587, 1429, 1102, 756 and 637 cm^{-1}. **UV** l**max (MeOH)** 206, 231, 278 and 309nm. **EIMS m/z (relative intensity, %):** 278 (M+-Cl, $C_{14}H_{18}N_2O_4$, 7), 151 (2), 142 (47), 141 (100), 137 (11), 127 (3) and 109 (6). **1H-NMR (D6, DMSO, 300 MHz)** d: 1.51 (m, 1H, H-4), 1.61 (m, 4H, H-3, 5), 2.03 (m, 4H, H-2, 6), 3.62 (s, 2H, H-1′′), 6.72 (s, 1H, OH), 6.79 (d, J=8.14 Hz, 1H, H-5′), 7.38 (dd, J=1.93 Hz, 5.3 Hz, 1H, H-6′), 7.42 (d, J=2.72 Hz, 1H, H-2′), 7.21 (s, 1H, H-OH). δ 6.6 Hz (s, 1H-$CONH_2$), δ 7.1 Hz (s, 1H-$CONH_2$).

4-Carbamoyl-1-[2-(3-nitrophenyl)-2-oxoethyl]-piperidinium bromide (III)

Off-white shiny crystals obtained from aqueous methanol (40%), **m.p**. 2443-244°C. **IR,** m**max** 3370, 3180, 2920, 1700, 1650, 1520, 1345, 820, 730 and 670cm^{-1}. **UV** l**max (MeOH)** 193, 199 and 229nm. **EIMS m/z (relative intensity, %):** 291 (M+-Br, $C_{14}H_{17}N_3O_4$, 12), 169 (3), 164 (4), 150 (8), 147 (46), 141 (100) and 127 (4). **1H-NMR (DMSO, 400 MHz)** d: 2.49 (m, 1H, H-4), 2.54 (m, 4H, H-3, 5), 3.15 (m, 4H, H-2, 6), 5.19 (s, 1H, H-2′′), 7.90-7.96 (dd, J-7.3 Hz, 10.8 Hz, 1H, H-5′), 8.42 (d, J=9.2 Hz, 1H, H-4′), 8.57 (d, J=2.8 Hz, 1H, H-6′), 8.6 (d, J=1.2 Hz, 1H, H-2′). δ 6.6 Hz (s, 1H-$CONH_2$), δ 7.1 Hz (s, 1H-$CONH_2$).

4-Carbamoyl-1-[2-(4-nitrophenyl)-2-oxoethyl]-piperidinium bromide (IV)

Mustard crystalline powder obtained from aqueous methanol (45%), m.p. 205-210°C. IR, µmax 3386,

3268, 3199, 2941, 1666, 1519, 1384, 853 and 610 cm^{-1}, UV λmax (MeOH) 203 and 262nm. EIMS m/z (relative intensity, %): 291 (M+-Br, $C_{14}H_{17}N_3O_4$, 4), 189 (2), 150 (5), 122 (2), 142 (3), 141 (100), 127 (17) and 109 (2). 1H-NMR (DMSO, 400 MHz) δ: 2.02 (2H, m, H-5), 2.21 (1H, m, H-4), 2.96 (2H, m, H-3), 3.30 (2H, m, H-6), 3.42 (2H, m, H-2), 6.57 (2H, s, H-2′′), 8.28 (2H, d, J=8.69 Hz, H-2′, 6′), 8.37 (2H, d, J=8.69 Hz, H-3′, 5′). δ 6.6 Hz (s, 1H-$CONH_2$), δ 7.1 Hz (s, 1H-$CONH_2$).

4-Carbamoyl-1-[4-bromophenyl)-2-oxoethyl]-piperidinium bromide (V)

Creamy crystalline powder obtained from aqueous methanol (72%), **m.p**. 246°C. **IR,** m**max** 3414, 3182, 2949, 1691, 585, 1397, 1229, 968, 809, 649 and 569 cm^{-1}, **UV** l **max (MeOH)** 203 and 262nm. **EIMS m/z (relative intensity, %):** 325 (M+-Br, $C_{14}H_{16}N_2O_2Br$, 36), 197 (6), 183 (53), 155 (4), 169 (2), 142 (37), 141 (100) and 127 (6). **1H-NMR (DMSO, 400 MHz)** d**:** 2.13 (2H, m, H-5), 2.10 (1H, m, H-4), 2.74 (2H, m, H-3), 2.95 (2H, m, H-6), 3.27 (2H, m, H-2), 6.51 (2H, s, H-2′′), 7.25 (2H, t, J=9.02 Hz, H-3′, 5′), 8.22 (2H, d, J=9.04 Hz, H-2′, 6′). δ 6.6 Hz (s, 1H-$CONH_2$), δ 7.1 Hz (s, 1H-$CONH_2$).

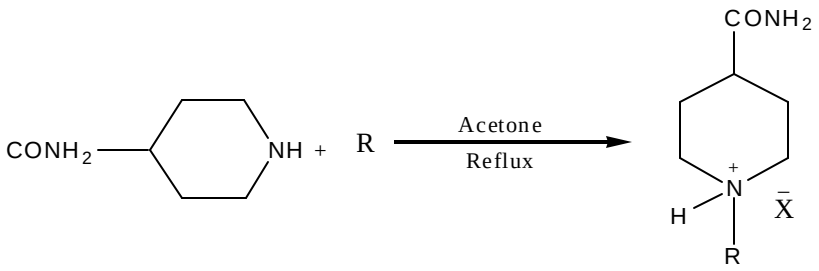
4-Carbamoyl-1-(2,4-dioxo-1,2,3,4-tetrahydro-pyrimidin-5-ylmethyl)- piperidinium Chloride (XIII)

Dull white powder obtained from aqueous methanol (13%**), m.p. 285-290°C IR** m**max**:: 3525, 3400, 2925, 2675, 1660, 1620, 1445, 1325, 750 and 680 cm^{-1}. **UV** l_{max} **(MeOH) nm**: 204 and 244. **EIMS *m/z* (relative intensity, %):** 260 (M^+-Cl, $C_{11}H_{15}N_2O_2$, 27), 182 (4), 154 (5), 142 (5), 133 (10), 127 (3) and 119 (4). **^{1}H-NMR (DMSO, 300 MHz)** d**:** 1.56 (m, 4H, *H*-3, 5), 1.63 (m, 1H, *H*-4), 2.01 (m, 4H, *H*-2, 6), 3.1 (s, 2H, *H*-1′′), 5.4 (s, 2H, *H*-1′), 6.3 (δ 6.6 Hz (s, 1H-$CONH_2$), δ 7.1 Hz (s, 1H-$CONH_2$).

4-Carbamoyl-1-(3-oxo-3-phenyl-propyl)-piperidinium chloride (XIV)

White fine shiny needle-like crystals obtained from aqueous methanol (74%)

mp: 158-162°C**. IR** m**max:** 3525, 3400, 2925, 2675, 1660, 1620, 1445, 1325, 750 and 680. cm^{-1} **UV** l_{max} **(MeOH)**: 204 and 244 nm. **EIMS *m/z* (relative intensity, %):** 260 (M^+-Cl, $C_{15}H_{20}N_2O_2$, 27), 182 (4), 154 (5), 142 (5), 133 (10), 127 (3) and 119 (4). **^{1}H-NMR (DMSO, 300 MHz)** d: 3.55 (m, 1H, *H*-4), 1.92-2.15 (m, 4H, *H*-3, 5), 3.61 (m, 4H, *H*-2, 6), 3.64 (m, 4H, *H*-1′′, 2′′), 7.65 (m, 1H, *H*-4′), 7.54 (m, 2H, *H*-3′, 5′), 8.06 (m, 2H, *H*-2′, 6′), δ 6.6 Hz (s, 1H-$CONH_2$), δ 7.1 Hz (s, 1H-$CONH_2$).



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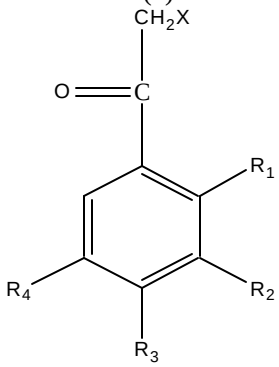
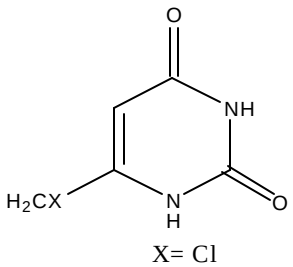
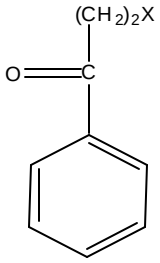


(II) R_1=R_4=H, R_2=R_3=OH, X=Cl,
(III) R1=R3=R4=H, R_2=NO_2, X=Br,
(IV) R1=R2=R4=H, R_3=NO_2, X=Br
(V) R_1=R_2=R_4=H, R_3=Br, X=Br



X= Cl

(XIII)



X= Cl

(XIV)

RESULTS AND DISCUSSION

Six newly synthesized compounds (II-V, XIII & XIV) along with their precursor (hexahydroisonicotinamide i.e.isonipecotamide, I) were subjected to study the effects on mean arterial blood pressure. The results of cardiovascular activities (blood pressure and heart rate) of these compounds were presented in Table-1 and Figs. 1-3. Effects on blood pressure were given by percent change.

From the table, it was apparent that the parent compound, hexahydroisonicotinamide (I) was found to be hypotensive and caused 35% reduction in blood pressure. Among all the derivatives, three compounds (II, III, & XIV) had been found to be hypertensive, one was hypotensive and the remaining two were found to be inactive at the tested dose but none of the compounds was proved to be more potent than the parent compound, (I).

During structure activity studies of these compounds, the nitro derivatives (III & IV) elicited different activities only by changing the position of nitro group. The compound III having nitro group at *meta* position caused 12% increase in blood pressure but by changing the position of nitro group from *meta* to *para*, the activity was reversed. Hence, compound IV i.e., 4-carbamoyl-1-[2-(4-nitrophenyl)-2-oxoethyl]-piperidinium bromide showed hypotensive activity, though this activity was not significant than that of the control. When bromo group was substituted at *para* position in the same compound, activity was completely lost, and this compound (V) had no effect on the blood pressure. Compound XIV i.e., the phenylpropyl derivative of isonipecotamide showed hypertension. The hypertensive activity of this compound and that of compound III were found to be equipotent. The derivative 4carbamoyl-1-[2-(4'-dihydroyphenyl)-2-oxoethyl]-piperidinium chloride (II) had produced mild hypertension i.e., only 9.6%.

Table 1: Effect of phenacyl derivatives of hexahydroisonicotinamide on mean arterial blood pressue (MABP) in normotensive anaesthetized rats

Compound	Concen-tration	Mean arterial blood pressure (MABP) (mm Hg)			Response	Heart Rate
		Control	Test	% Change		
I	10^{-2}M	120.0	78.0	–15	Hypotensive	0
II	″	118.0	129.3	+9.6	Hypertensive	0
III	″	116.0	130.7	+12	Hypertensive	0
IV	″	120.0	95.0	–21	Hypotensive	0
V	″	116.05	116.0	0	Inactive	0
XIII	″	114.7	114.7	0	Inactive	0
XIV	″	116.0	130.7	+12	Hypertensive	0

(+) Increase in B.P. (–) Decrease in B.P.

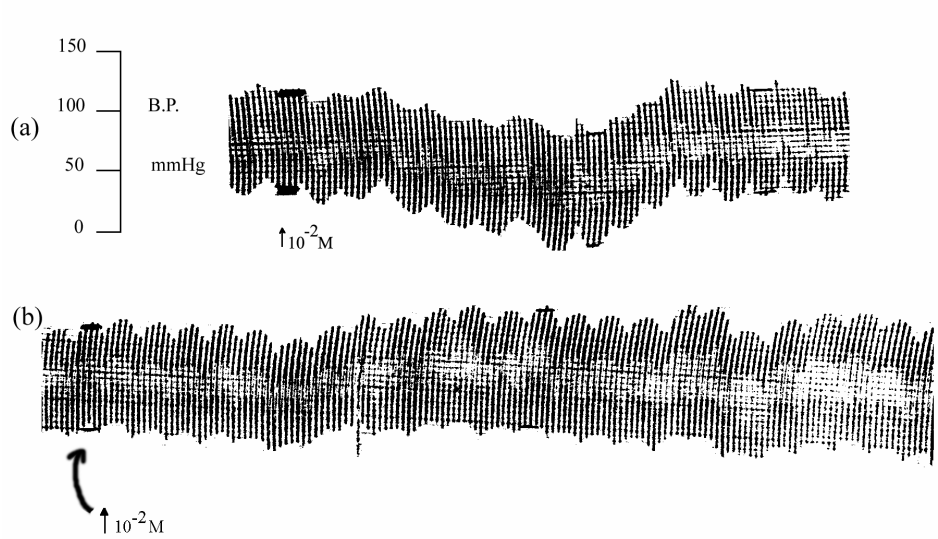


Fig.1: Tracings showing effects of (a) hexahydroisonicotinamide (I) and (b) its derivative (II) on blood pressure in Normotensive anaesthetized rats (arrows indicating the administration of compounds)

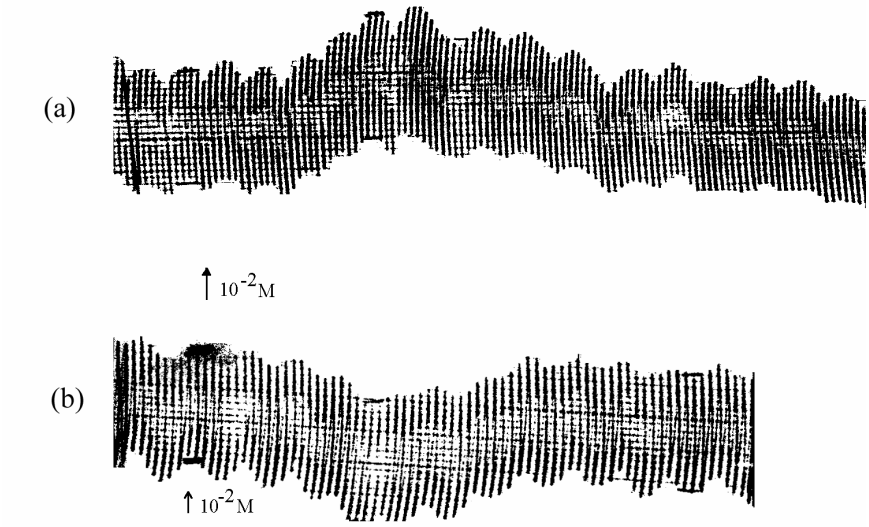


Fig.2: tracings showing effects of hexahydroisonicotinamide derivatives (a) (III) and b (IV) on blood pressure in Normotensive anaesthetized rats (arrows indicating the administration of compounds).

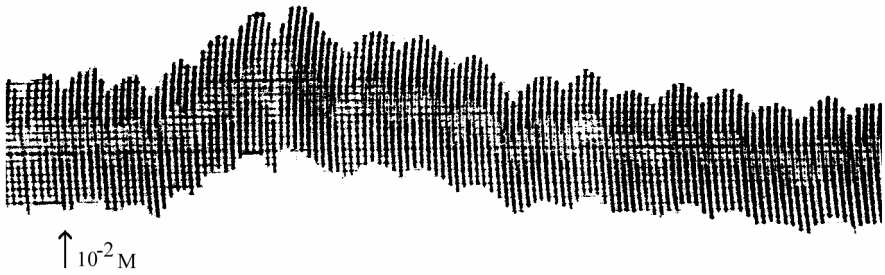


Fig.3: Tracings showing effects of hexahydroisonicotinamide derivatives (XIV) on blood pressure in Normotensive anaesthetized rats (arrows indicating the administration of compounds)

Almost similar types of derivatives synthesized from nipecotamide were reported by one of the research students .[27] It had been found that both nipecotamide and isonipecotamide in which the only difference was the position of carboxamide group, were hypotenisve but the intensity of hypotension was different. Isonipecotamide was only 35% hypotensive while nipecotamide was 63%. By comparing the derivatives of nipecotamide and isonipecotamide, it can be observed that effects of these compounds in most of the cases were comparable. For example, 3, 4-dihydroxy compound (derivative of isonipecotamide) was hypertensive while the same compound synthesized from nipecotamide was hypotensive. Hence it can be predicted that the position of carboxamide group in the piperidine ring was important for its activity. In case of nitro derivatives, nitro group at *para*-position in both the cases caused no difference in activity. Similarly, bromo derivatives of both of these starting compounds had been found to be inactive. These findings suggest that position of carboxamide group in the heterocycle has not considerable effects on arterial blood pressure and playing no important role in the increase or decrease of blood pressure.

Present study demonstrated that the intravenous administration of all the above-mentioned compounds induced hypertension in some cases and hypotension in others without affecting the heart rate of animals. The mechanism underlying decrease or increase in blood pressure for these compounds cannot be fully established only on the basis of this study. However, by comparing the effects with that of standard drugs, the mechanism can be predicted. Moreover, certain facts are important to understand the effects on MABP and can be described in this way.

Mean arterial blood pressure (blood pressure) is the product of cardiac output and peripheral vascular resistance and the maintenance of normal blood pressure is dependent on these parameters. However, cardiac output and peripheral, vascular resistance are modulated by sympathetic nervous system.[28] Blood pressure recorded by a

pressure transducer via polygraph in experimental animals such as rat shows two types of pressures i.e., systolic pressure and diastolic pressure. Left ventricle of heart contracts, as a result of which maximum pressure exerted by the blood on the arterial walls represent systolic pressure. Whereas, diastolic pressure represents the minimum pressure on the blood vessel walls, when the ventricle relaxes and refills with the blood. In normal adult man, the values of systolic and diastolic pressures are about 120 and 80 mmHg, respectively while the heart rate is 72beats/min.[29] Hypertension is an elevation of systolic and/or diastolic pressure to be above 140/90 mmHg. It is a multi-factorial disease frequently associated with other cardiovascular problems. Various drug classes are available for the treatment of hypertension e.g., ß-blockers, central agents and calcium channel blockers.[30]

In rats the normal values of mean systolic and diastolic blood pressure are 116 and 90 mmHg respectively whereas, the heart rate is 300beats/min.[31] In the present study a direct blood pressure measuring technique (i.e., cannulation method) was used to determine the effects of synthesized compounds on the cardiovascular parameters (blood pressure and heart rate) of anaesthetized rats.[32-33] To verify the experimental setup and also for comparison with the test compounds, standard drugs such as acetylcholine (hypotensive agent) and nor-adrenaline (hypertensive agent) were used. It is well established that acetylcholine produces hypotension by activating the muscarinic receptors located on the epithelium of blood vessels. Intravenous administration of acetylcholine elicits relaxation of vascular smooth muscles by releasing the endothelium derived relaxing factor (EDRF) from the intact endothelial cells.[34-35] Similarly, the activation of muscarinic receptor (M_2) in the heart produces decrease in heart rate.[36-37]

It is also well established that the normal tone of blood vessels is maintained through the activation of adrenoceptors (a_1 subtype) by releasing nor-adrenaline from innervated nerve endings discussed already by Rosendroff in 1986.[28] Therefore, alpha-1 blocking agents produce hypotension. Nor-adrenaline, being a potent vasoconstrictor mediates its effect through the activation of a_1-adrenoceptor.[38] The vasoconstrictor response of nor-adrenaline was not reduced significantly by these compounds rules out the possible involvement of alpha adrenoceptors.

In conclusion, among the tested compounds, three of them produced mild hypertension while only one was found to be hypotensive. This study is the preliminary investigation that can further be extended to explore the potentials of these compounds leading potent antihypertensive and/or hypotensive agents.

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