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Synthesis and Antihypertensive Screening of Novel Substituted 1,2-Pyrazoline Sulfonamide Derivatives

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ABSTRACT

The novel compounds containing two phenyl rings at 3, 5 position of 1, 2-pyrazoline sulphonamide derivatives synthesized. N-((3-(4-aminophenyl)-5-(3,4-disubstitutedphenyl)-4,5-dihydropyrazol-1-yl)methyl)benzenamine screened for Angiotensin II receptor antagonistic activity in 1K-1C goldblatt hypertensive rats using tail cuff method. N-((3-(4-aminophenyl)-5-(3,4-dichlorophenyl)-4,5-dihydropyrazol-1-yl)methyl)benzenamine sulphonamide exhibited potent antihypertensive activity.

Keywords: 1,2-pyrazoline, sulfonamide, angiotensin II, antihypertensive

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INTRODUCTION

The term hypertension is generally defined as pathologically elevated systemic arterial pressure. Although the number of cardiovascular deaths has decreased over the past 25 years, achieving long term control of hypertension in millions of patients remains as important objective[1,2].

Angiotensin II receptor antagonists, also known as angiotensin receptor blockers , AT_1-receptor antagonists or sartans, are a group of pharmaceuticals which modulate the renin-angiotensin-aldosterone system. Their main use is in hypertension, diabetic nephropathy and congestive heart failure[2,3,4]. These substances are AT_1-receptor antagonists which block the activation of angiotensin II AT_1 receptors. Blockade of AT_1 receptors directly causes vasodilation, reduces secretion of vasopressin, reduces production and secretion of aldosterone, amongst other actions the combined effect of which is reduction of blood pressure. Irbesartan is a safe and effective angiotensin II receptor antagonist with an affinity for the AT1 receptor that is more than 8,500 times greater than its affinity for AT2 receptor. This agent has a higher bioavailability (60-80%) than other drugs in its class[3]. In both Losartan and Irbesartan structures imidazole moiety is being present. A structure analog of losartan and Irbesartan are designed by incorporating the heterocycles like pyrazoline group. We felt it would be interesting to explore the possibilities of 1,2-pyrazoline derivatives for Angiotensin II receptor antagonistic activity. The Irbesartan structure was a modified Losartan structure, which had all the identity of a Losartan molecule but with groups that would fit the hydrophobic cavity with a tetramethylene group and an alkyl side chain that would fit in the pocket in the AT1 receptor. The hydroxyl methyl group of Losartan being replaced with carbonyl group of Irbesartan[4,5,6]. With a view to introduce a hydrogen bonding interaction with AT1 receptor, these structures were further modified with a view of retaining both hydrogen bonding characteristics and as well as lipophilic groups. Losartan and Irbesartan structure contains a diphenyl molecule & imidazole ring. In Losartan and Irbesartan diphenyl molecule is attached to the nitrogen of the imidazole ring. It is interesting to to see the activity of compounds containing two phenyl rings attached at two different positions namely 3, 5 position of 1, 2-pyrazoline ring. The sulphonamide derivatives known for its diuretics activity which reduces renal hypertension[5,6,7]. We use to synthesize sulphonamide and pyrazoline in one molecule to check its possible Angiotensin II receptor antagonist property. For this reason chalcones were synthesized reacted with hydrazine hydrate to yield the corresponding 1,2-pyrazoline derivatives which further condensed with sulphanilamide and formaldehyde by mannich condensation reaction [8,9,10].

MATERIALS AND METHODS

Melting points of the synthesized compounds were determined by open capillary tube method and were uncorrected. TLC was performed on microscopic slides (2×7.5cm) coated with Silica-Gel-G and spots were visualized by exposure to iodine vapor. IR spectra of all compounds were recorded on FT-IR 8400S Shimadzu Prestige 21 spectrophotometer using KBr disc method. ^{1}HNMR spectra were recorded on Bruker-NMR- av 300, DMSO as internal standard. The instrument is equipped with a cryomagnet of field strength 9.4 T. Its ^{1}H frequency is 300 MHz. The Mass spectra were recorded on GCMS – SHIMADZU-PC 391.

Procedure for synthesis of chalcones [11,12,13]:

To a solution of sodium hydroxide (22gm) in water (200 ml), ethanol (122.5 ml) was added and the flask was immersed in a bath of crushed ice. Equimolar quantity, 0.43M, of both aldehyde and p- acetamido acetophenone were added to the above mixture with continuous stirring. Stirring was carried, for about 3-4 hr. The reaction mixture was kept in the ice chest for 24-hr and the solid obtained was filtered and washed using cold water until all the washings were neutral to litmus. The crude product obtained was recrystallized from alcohol.

procedure for the synthesis of 3,5-diaryl 1,2- Pyrazolines [14,15,16]:

A mixture of chalcone (0.01 M) and hydrazine hydrate (0.02 M) in ethanol (20 ml) was taken in an RBF. The reaction mixture was refluxed for 6-8 hrs. The reaction mixture was monitored using TLC solvent system Benzene: Ethyl acetate – 9: 1 and purity was confirmed by single spot. The resulting solution was left overnight in a refrigerator, the crystals separated were used for the next step immediately.

procedure for the synthesis of Mannich base [17,18,19]:

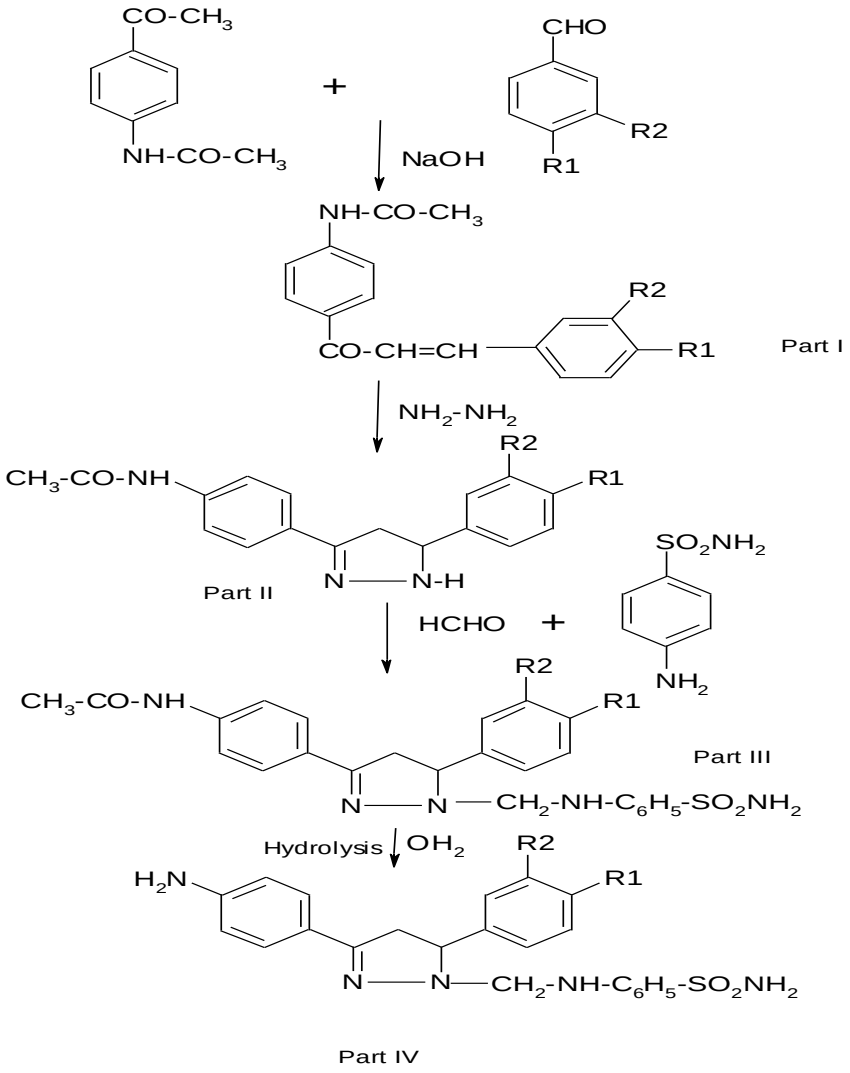
A mixture of Pyrazoline (0.02 mol) and Sulphanilamide (0.02 mol) in Methanol (40 ml) was taken in an RBF. Then Paraformaldehyde (0.01 mol) and one drop of concentrated HCL was added and the reaction mixture was refluxed for 3-4 hrs. On cooling the reaction mixture and reducing the volume by vacuum suction the product separated out which was filtered and crystallized from Methanol.

Hydrolysis of P-acetamido group of Mannich base [20,21,22]:

1 gm of Mannich base, 35 ml of Methanol was taken in RBF with reflux condenser, the resulting solution was heated to boiling then with pressure equalizing dropping funnel added 1.22 ml of concentrated HCl drop by drop . It was refluxed for 30-40 min or until test portion remains clear upon diluted with water. Then dilute with 150 ml of water and fit flask set for downward

distillation. Distilled mixture from air bath and collected about 100 ml distillate. Poured residual solution into 100 ml ice-water and added with vigorous stirring 5% Sodium hydroxide solution until just alkaline. Crystals separated are washed on buckner with cold water and dry in air upon pads of filter paper. Recrystalized from ethanol. Synthetic data is represented in Table 1 and Table 2.

Scheme :



Pharmacological screening [23,24,25]:

Swiss Albino mice of either sex weighing about 20-25 gm were taken for the toxicity and male Albino Wistar rats weighing about 210-240 gm were used for assessing antihypertensive activity.

Acute Toxicity Study (LD_{50}) [26,27,28] :

This study was carried out in order to establish the therapeutic and toxic doses of the newly synthesized 1,2 pyrazoline derivatives. To establish LD_{50} of these compounds the method described by Miller & Tainter was employed.

Induction of experimental hypertension [29,30,31] :

Albino rats weighing 200-250 gm were anaesthetized with anaesthetic ether. The fur on the back was shaved and the skin was disinfected. In the left lumbar area flank incision was made parallel to a long axis of the rat. The renal pedicel was exposed with the kidney retracted to the abdomen. The renal artery was located and a u-shaped silver clip was clipped around it near to the aorta using special forceps, the size of the clip was adjusted so that the internal gap ranges from 0.25 – 0.38 mm. the right kidney was removed after tying of the renal pedicle. The skin incisions were closed and appropriate treatment was given to prevent infection. Blood pressure was measured 4-5 weeks after clipping and rats with values higher than 150 mm Hg were selected for experiments. Blood pressure readings were taken on each of 3 days prior to drug treatment. Rats are divided into 6 animals per dose and each animal is used as its own control. One of the groups will serve as standard and given 27 mg/kg bodyweight of losartan. Initial reading (prior) and 1 hr post-drug blood pressure readings were taken.

Measurement of systolic blood pressure [32,33,34]:

The systolic blood pressure is measured by tail cuff method by Harvard non invasive BP apparatus. The acquisition of data was done by bio-pack data acquisition system and visualized on the computer screen. Tail cuff method is a common and convenient means to measure systolic blood pressure in rats. The tail cuff is inflated and then deflated. Pulsations disappear when cuff is inflated. When cuff starts deflating pulsations start appearing when pressure in the cuff equals systolic pressure. The cuff is attached to Harvard non invasive BP monitor and BP is recorded. The results were analyzed by one-way ANOVA followed by Dunnet's test (p-value ≤ 0.05). The results are depicted in Table 3 and Graph 1.

RESULTS AND DISCUSSION

We report new 1,2 pyrazolines which defined combination of substituents confers appropriate polarity and charge distribution for good activity. The spectral data of compounds confirms the structures.

Spectral data of synthesized n-((3-(4-aminophenyl)-5-(3,4-dichlorophenyl)-4,5-dihydropyrazol-1-yl)methyl)benzenamine sulphonamide derivatives:

IR(Cm^{-1}): Compound PH-1: 831, 3464 ($-NH_2$) 1174 (S=O) 1338 ($-CH_2$) 1448 (C=N) 1517, 1600 (Ar- C-C) 3217 (Ar-H) 3346 (-NH). Compound PH-2: 831, 3448 ($-NH_2$)1174 (S=O) 1313 (CH_2) 1436 (C=N)1517, 1595 (Ar- C-C) 3219 (Ar-H) 3350 675,1012 (-Cl). ;Mass *m/z* (% abundance) 456 [M^+](100%) , 457.9 [M^++2] (31.2%)

Compound PH-3: 830, 3360 (-NH_2) 1130, 1180 (S=O) 1230, 3240 (-NH) 1320 (-CH_2) 1440 (C=N) 1520, 1600 (Ar- C-C) 2960 (Ar -CH).

Compound PH-4: 607 (C-Br) 831, 3367(-NH_2) 1174 (S=O)1319 (-CH_2)1436 (C=N) 1516, 1595(Ar- C-C) 3334 (-NH) .

Compound PH-5: 840, 3360 (-NH_2) 1120, 1180 (S=O) 3220 (-NH)1350 (-CH_2) 1450 (C=N) 1520, 1600 (Ar- C-C) 1320 (Ar-NO_2).

Compound PH-6: 840, 3360 (R-NH_2)1100, 1180 (S=O) 3240 (-NH)1350 (-CH_2)1440 (C=N) 1500,1600 (Ar- C-C)1300(C-OCH_3)

Compound PH-7: 830, 3360(-NH_2) 1130, 1180 (S=O) 3240 (-NH) 1360 (-CH_2) 1440 (C=N) 1520, 1600 (Ar- C-C) 800 (C-Cl) . ;^{1}H NMR (ppm): 2.0 - 2.3(2H, d, -CH_2 of –CH_2-NH) 3.9 (H, s, -CH of Pyrazoilne ring) 5.3 – 5.6 (2H, d, -CH_2 of Pyrazoline ring) 6.0(2H, s, -NH_2 of –SO_2NH_2) 6.2 – 6.3 (H, d, -NH of Ar-NH-CH_2) 6.5 – 6.6(2H, d, 2H of Aromatic protons) 7.0 (1H, s, 1H of Aromatic protons) 7.3 – 7.8 (8H, m, 8H of Aromatic protons)7.9 – 8.0 (2H, d, -NH_2 of Ar-NH_2).

Compound PH-8: 3440 (Ar- OH) 840, 3350 (-NH_2) 1130, 1180(S=O) 3240 (-NH) 1360 (-CH_2) 1440 (C=N) 1520, 1590 (Ar- C-C)

We also report various derivatives of 1,2 pyrazolines from Chalcones to achieve the promising and selective inhibition. The synthesized 1,2 pyrazoline derivatives have resemblance to some of Angiotensin II blocking agents like Irbesartan and Losartan. Hence it was thought of carrying out Angiotensin II blocking screening by 1K-1C Gold blatt non invasive blood pressure measurement method in the department of pharmacology K.L.E.U's College of Pharmacy, Belgaum. As a result, the screened compounds have shown significant Angiotensin II blocking activity. Irbesartan was used as the standard drug for Angiotensin II blocking activity. Compounds synthesized have shown the significant Angiotensin II blocking activity when compared with the control but however are not comparable to the standard Irbesartan. The results were calculated by taking mean ±SE and finding the 'P' values. Hence from the SAR studies the chloro substituted compounds PH-2 and PH-7 exhibit better Angiotensin II blocking activity amongst the synthesized compounds. The structures of all the synthesized compounds were established on the basis of M.P., TLC, IR, NMR (PMR) . The compounds synthesized were found to be non-toxic and could be synthesized in good yields. The active compounds could be taken as lead for structural and molecular modification was thought of in future.

Table 1: List of synthesized Compounds

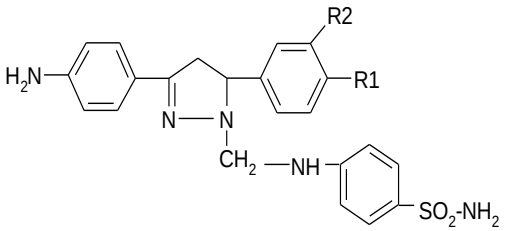
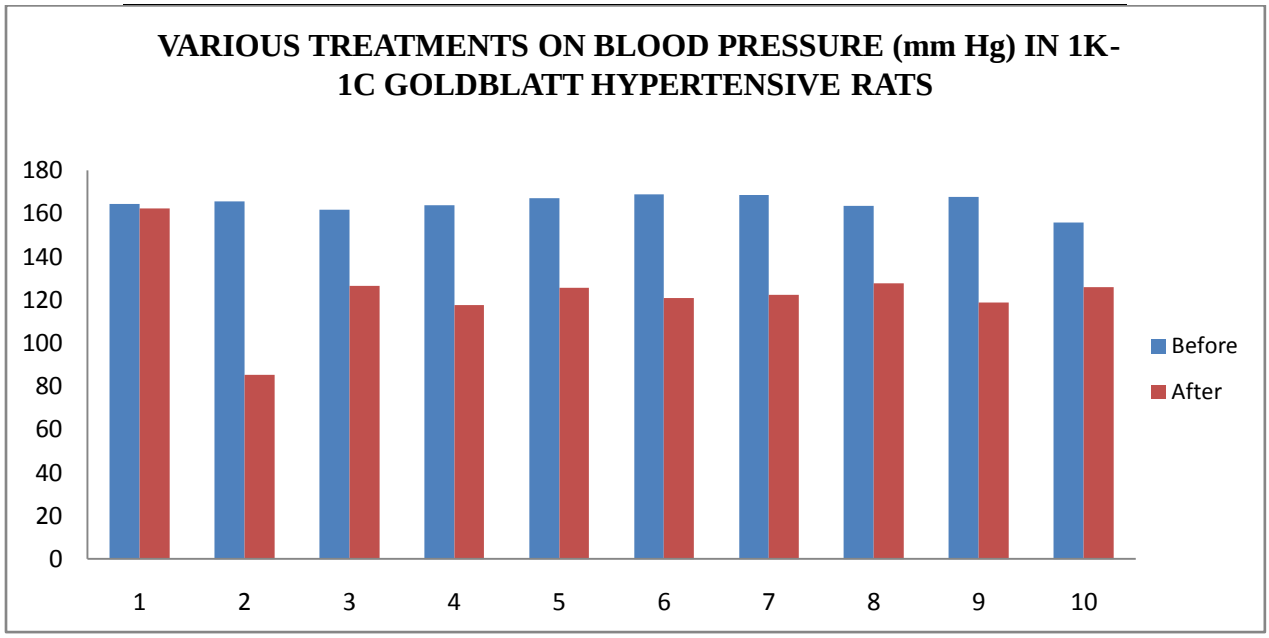
Sr. No.	Compounds	Comp.code	R1	R2
1		PH1	H	H
2		PH2	Cl	H
3		PH3	CH_3	H
4		PH4	Br	H
5		PH5	NO_2	H
6		PH6	OCH_3	H
7		PH7	Cl	Cl
8		PH8	OH	H

Table 2: Analytical data of the synthesized compounds

Com.	Mol. Formula	Mol Wt.	M.P. ^{0}c	Yield%	R_f
PH1	$C_{22}H_{23}N_5O_2S$	421.5	88-90	49.6	0.71
PH2	$C_{22}H_{22}N_5O_2SCl$	455.9	100-102	46.4	0.86
PH3	$C_{23}H_{25}N_5O_2S$	435.5	150-152	42.5	0.74
PH4	$C_{22}H_{22}N_5O_2SBr$	500.4	96-98	40.1	0.76
PH5	$C_{22}H_{22}N_6O_4S$	466.5	136-138	53.3	0.82
PH6	$C_{23}H_{25}N_5O_3S$	451.5	104-106	51.2	0.84
PH7	$C_{22}H_{21}N_5O_2SCl_2$	490.4	108-110	54.6	0.78
PH8	$C_{22}H_{23}N_5O_3S$	437.5	120-122	46.6	0.72



Graph 1 Various Treatments On Blood Pressure (mm Hg) In 1K-1C Goldblatt Hypertensive Rats

Where,

On Y-axis : Mean Blood Pressure

On X-axis : Treatment-

1.Control, 2. Irbesartan, 3.PH-1, 4.PH-2, 5.PH-3,6.PH-4,7.PH-5,8.PH-6,9.PH-7,10.PH-8

All shows P value < 0.01

Table 3:Effect on B. P. IN 1K-1C goldblatt hypertensive rats:

Group No.	Drug and Dose (mg per kg bodyweight)	Mean blood pressure in mm of HgMean ± SE		P value
		Before Treatment	After Treatment	
1	Control	164.4 ± 2.112	162.2 ± 1.461	---
2	Irbesartan	165.6 ± 2.531	85.2 ± 3.567	<0.01
3	PH 1	161.7 ± 3.412	126.4 ± 3.478	<0.01
4	PH 2	163.8 ± 2.542	117.5 ± 2.972	<0.01
5	PH 3	166.9 ± 3.332	125.6 ± 2.836	<0.01
6	PH 4	168.7 ± 4.211	120.8 ± 4.962	<0.01
7	PH 5	168.5 ± 3.643	122.3 ± 2.052	<0.01
8	PH 6	163.3 ± 3.467	127.5 ± 2.869	<0.01
9	PH 7	167.6 ± 2.342	118.6 ± 3.985	<0.01
10	PH 8	55.8±2.332	125.7 ± 1.492	<0.01

CONCLUSION:

The present work, which was undertaken is a bonafide and novel work on the synthesis of 1,2 pyrazoline derivatives. We have made an attempt in reviewing the literature on 1,2 pyrazoline derivatives for their medicinal uses with the help of chemical abstract, journals and internet surfing. For the synthesis of 1,2 pyrazoline derivatives scheme were established. Around eight derivatives were synthesized. The synthesized compounds were tested for their purity, preliminary tests, physical constants and TLC. The structure of the final compound were confirmed by IR, ^{1}H-NMR spectra and GCMS. The compounds synthesized were found to be non-toxic and could be synthesized in good yields. The proposed compounds were screened for their Angiotensin II blocking activity against the standard drug Irbesartan. Compounds synthesized have shown the significant Angiotensin II blocking activity when compared with the control but however are not comparable to the standard Irbesartan. Hence from the SAR studies the chloro substituted compounds PH-2 and PH-7 exhibit better Angiotensin II blocking activity amongst the synthesized compounds. The active compounds were taken as lead and structural and molecular modification was thought of in future. The present work is an attempt in this direction and the efforts have proved to be quite fruitful and promising. It will be worthwhile to concentrate on molecular manipulation of the central 1,2 pyrazoline derivatives moiety and also its SAR and QSAR studies.

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