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DESIGN, SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF SOME NOVAL POTENT ANGIOTENSIN II RECEPTOR ANTAGONIST WITH ANTIHYPERTENSIVE ACTIVITY

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ABSTRACT

Intervension of Renin Angiotensin Aldosterone system by a comparatively newer class of antihypertensive agents, Angiotensin II receptor blockers (ARB), have proved to be very effective for treatment of Hypertension.Accordingly, in the current work,some *novel angiotensin II type 1 receptor antagonists have been designed and synthesized.The synthesized derivatives were further subjected to pharmacological evaluation.The Anti-hypertensive activity of the derivatives was determined by Invasive method on Angiotensin II induced hypertensive rats .The activity was also determined using non invasive method of blood pressure measurement by rat tail cuff.Good results were obtained by both of the methods.The antihypertensive effect of the derivatives was maintained for a considerable period of time , which indicated that they have favorable blood pressure-lowering properties.* Experimental findings were in good agreement with docking studies.T*he proposed derivatives can therefore be considered as novel anti-hypertensive candidates and deserve further investigations*.

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Keywords- AT1,AT2,Antagonist, Angiotensin *etc.*

INTRODUCTION

The renin-angiotensin-aldosterone system (RAAS) is an important regulator of blood Pressure .It also has a role in the fluid and electrolyte homeostasis, pathogenesis of hypertension, congestive heart failure and chronic renal failure. It produces Angiotensin II (Ang II), a very potent vasoconstrictive and volume-retaining hormone which is partly responsible for regulation and maintenance of blood pressure[1] . A recent report showed mast cells as an additional source of renin constituting a unique extra renal renin-angiotensin system.The actions of angiotensin II, an octapeptide and a mediator of RAAS, are

mediated through specific surface receptors present on the various target organs . According to current nomenclature, these receptors are classified into two subtypes viz. AT1 and AT2 . Majority of physiological actions of Ang II are mediated through the AT1 Receptors.These are vasoconstriction, release of aldosterone and vasopressin, retension of sodium and water in cell proliferation,left ventricular hypertrophy, nephrosclerosis, vascular media hypertrophy, endothelial dysfunction, neointima formation and processes leading to athero-thrombosis[2].

Although AT1 receptors predominate in most of the tissues, AT2 receptors are widely expressed in fetal tissue, uterus, adrenal medullary tissue and in discrete parts of the brain of various species, including humans[3]. Ang II also stimulates a cellular mitogenic response via the AT1 receptor . AT2 receptor fine-tunes the regulation of natriuresis,body temperature, blood pressure, reproduction, embryonic development, cell differentiation, tissue repair and programmed cell death.

In order to achieve the goals of managing hypertension, continues efforts have been made to intervene RAAS at specific sites . Three major sites of intervension are inhibition of Renin, inhibition of ACE and antagonism of Ang II . Renin, identified by Tigerstedt and Bergmann in 1898, is a specific and rate-limiting enzyme for ang II formation[4]. Several renin inhibitors were developed but they had few short comings.The major being poor bioavailability due to their peptide nature[5].At present, aliskiren is the first non-peptide orally active renin inhibitor to be approved by FDA.

Blockers of the RAAS such as angiotension converting enzyme (ACE) inhibitors have successfully been introduced into the market for the treatment of hypertension ,congestive heart failure , [6]coronary artery diseases ,diabetic nephropathy and to reduce proteinuria in chronic renal diseases .However, their use is associated with frequent side effects such as cough and angio-oedema due to bradykinin accumulation . [7]

With the development of a series of low molecular weight nonpeptide imidazole analogues with weak but selective, competitive AT1 receptor blocking property, [8] it became more and more apperent that blocking RAAS with specific antagonists of Ang II may turn out to be a more suitable way for blood pressure control. Many drugs came after structural modification of these lead compounds i.e.losartan ,eprosartan,valsartan,irbesartan,candesartan,telmi sartan,zolasartan,olmesartan and saprisartan.Most promising amongst all is Losartan .It is an orally active, nonpeptide, Ang II receptor Antagonist[9](DuP-753, 2-n-Buty-4-chloro-5-hydroxymethy1-1-[(2'-(1H-tetrazol-5-y1)biphenyl-4-y1)methy1)imidazole potassium salt. Many replacements of the imidazole part of Losartan by other heterocycles have been published in literature and patents. [10,11] Peter Buhlmayer and co-workers designed a novel series of orally active derivatives ,in which the heterocycle of losartan has been replaced by an acylated aminoacid. As per their finding the nature of the amino acid side chain was crucial for activity. High potency was achieved with several compounds derived from aliphatic amino acids.The findings demonstrate that good activity can also be achieved with amides derivatives of this unique structure and to a lesser extent with alcohol derivatives too[12] .The compound Valsartan of this series was found to have best activity in vivo and in vitro.Valsartan has a unique structure compared to all other AngII receptor antagonists.It was found to have the best potency efficacy and longest duration of action (up to 24 hrs) . Cosidering the importance of above findings about Valsartan,in the present work an attempt has been made to design and synthesize a series of novel *angiotensin II type 1 receptor antagonists.Homology modeling was done and docking calculations have also been performed using soft ware* Schrodinger *for the designed compounds.*

MATERIALS AND METHOD

Since there were no specific templates for homology modeling of angiotensin receptor 1,hence a threading*[13]*(fold recognition)approach was adopted.Accordingly, protein is modeled based

on same fold pattern searching as the protein of known structure. Threading works by using statistical knowledge of the relationship between the structures deposited in the PDB and the sequence of the protein. Protein sequence was submitted to I-Tasser server which models protein, based on multiple threading alignments by LOMETS and iterative TASSER assembly simulations*[14]*. Best model was selected on the basis of confidence score and TM-score of protein models. Confidence score lies in the range of [-5,2] and the calculations are based on the significance of threading template alignments and the convergence parameters of the structure assembly simulations. Further loop refinement of models was done by ModLoop, a web server for automated modeling of loops in protein structures*[15]*.Finally structure verification was done by ERRAT plot*[16]* version 2.0 and RAMPAGE.

Ligand preparation: The ligand molecules structure were constructed using Chem draw module of Chem sketch and transferred to chem 3D to convert them into 3D structures. The energy minimization of the molecules was done using MM2 force field followed by semi empirical AM1 (Austin model) Hamiltonian method available in MOPAC module, by fixing root mean square gradient as 0.1 and 0.0001 kcal/Mol respectively, for calculating partial atomic charges and electron density on various atoms. Geometry optimization of the proposed ligands was done by ligprep module of Schrodinger. The protonation states of all of the ionizable molecules of ligands were predicted by ionizer provided in Ligprep wizard and energy was minimized (only hydrogen atoms) using the OPLS2005 force field. LigPrep was run with the Stereoizer set to determine chiralities from 3D structure.

Protein preparation: The protein model was imported into the Maestro module available in the Schrödinger package and the protein was further optimized using the Protein Preparation Wizard. This optimization includes adding hydrogen atoms, assigning correct bond orders and building di-sulfide bonds. The protonation states of all of the ionizable residues were predicted. An optimized structure model was energy minimized (only hydrogen atoms) using the OPLS2005 force field.

Glide, a grid-based exhaustive search algorithm, was used for all docking experiments. Glide uses a series of hierarchical filters to find possible ligand pose in the active site, and the program has the option to treat the ligand fully flexible or rigid during the docking run. In addition, glide provides three docking precision modes, namely, XP (extra precision), SP (Standard precision) and HTS (High-throughput screening) modes. Each mode is used in slightly different context, e.g., the HTS mode is used to screen a relatively large database (uses more restricted conformational sampling), the SP mode uses a softer scoring function that adapt at identifying ligands that have a reasonable propensity to bind in the receptor, and the XP mode uses a complete minimization, and scoring from large ensembles of docking poses (requires more CPU time).Thus this mode is specially used for top-ranked compounds. Glide uses an in-build docking scoring function resulting in a Glidescore (SP and XP).The compounds we proposed here have been rationalized based on their highest xp docking score. Most of them have a fairly high Glide XP docking score.Some of these analogues show higher scoring than losartan and valsartan also . **(See Table 1)**.

Table no 1 Highest Docking Scores of synthesized analogues

S.no	***Entry***	***Dock score***
1	***VH-6***	-9.75
2	***VH-7***	-9.26
3	***VH-8***	-10.17
4	***VH-9***	-9.03
5	***VH-10***	-11.08
6	***VALSARTAN***	-7.53

Results of docking studies done on the newly designed and synthesized compounds fairly corresponds to the earlier studies *[17,18]*done on ARBs. Earlier studies say that for a good pharmacological activity, interactions with recidues like LYS 199,SER 109 SER 105,PHE 182,TYR 184,THR 260 at the active site are important, specifically in case of Valsartan.The active site recidue LYS 199 forms H bond with the carboxylate oxygen of the ligand .Further LYS199 was also seen to form a bridge between two acidic moiets of carboxylate and tetrazole,,allowing acidic groups to form multiple H bonds .This leads to a more stabilized system and a better packing of valsartan .Findings of Tuccinardi*[19]* on the other hand suggest that the tetrazole ring did not appear to interact with any of the recidue found important in mutagenesis studies .

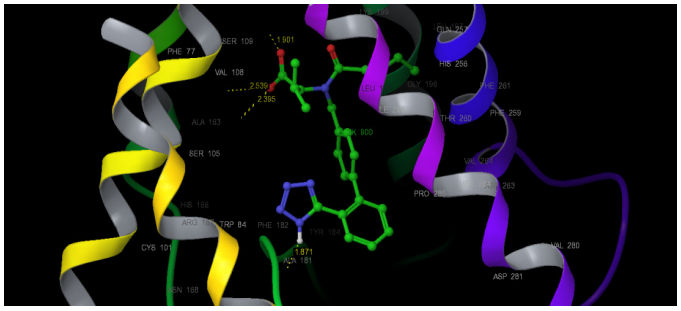


Figure 1. Docking pose of Valsartan at the active site of the AT1 receptor complex. Interatomic distances between H-bonded atoms are indicated in yellow.

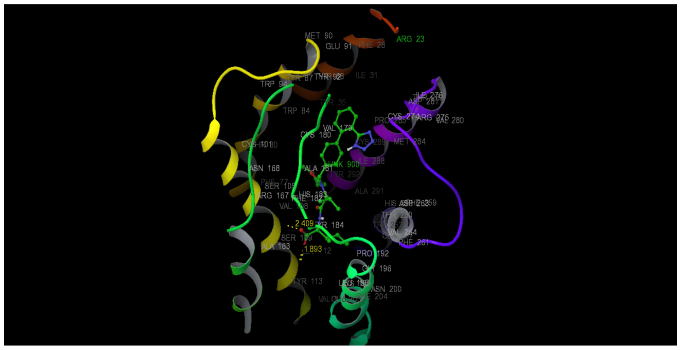


Figure 2. Top docking pose of compound VH-8 at the active site of the receptor. Interatomic distances between H-bonded atoms are indicated in yellow

Our findings with respect to interactions of tetrazole, corresponds to the above stated fact in most of the cases.Molecular docking of the proposed derivatives gave good results with higher Glide XP docking scores.

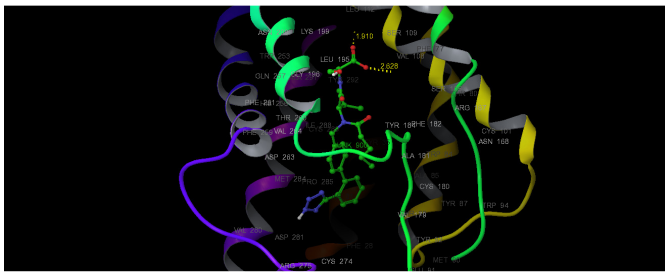


Figure 3. Top docking pose of compound VH-10 at the active site of the receptor. Interatomic distances between H-bonded atoms are indicated in yellow

Of them particularly better results were shown by VH-8 and VH-10.Details of the top docking poses of these two compounds are shown here as representative example **(See Fig.1,2 & 3).**

Corresponding amino acid residues for compound VH-8 are Lys199,Arg 167,Ser 109, Ser 105, Asn200, Thr260, Phe251,Val 280,Asp 281,Phe 257 and Ala 163.similarly in case of VH-10 close contacts are formed by Lys199,Arg167,Ser109,Ser105,Thr260,Val280 and Asp281.Molecular docking results obtained here are in good agreement with experimental findings too.These two facts together indicate that the proposed analogs have high binding affinity to the AT1 receptor.

Synthesis

In continuing endeavors to search novel and potent Ang II receptor Antagonist, , we have considered an important fact which says that nitrogen containing building blocks often play important roles in drug design and provide enhanced interaction between pharmacophore and receptor sites. Since amino acid recidues can often interact with the active site of receptors and play a pivotal role via H bond and charge effect, [20] here

synthesis of amino acid coupled derivatives were considered to be of specific interest .

*Accordingly in the present work, major chemical modification of our interest was synthesis of N terminal coupled amino acid derivatives . The required compouds VH-6 to VH-10 were prepared in four steps.The scheme of synthesis employed to synthesize the designed compounds is outlined in figure 4.In the first step natural amino acids 2(a-e) were converted to amino acid benzyl esters p-*toluene sulfonate 3(a-e) *. This* Conversion was accomplished by the treatment of respective amino acids with calculated amounts *p*-toluene sulfonic acid (PTSA)and benzyl alcohol .PTSA is added to Benzyl alcohol at room temperature .Reaction is endothermic and the temperature goes down to 3-4˚C.This is followed by addition of calculated amount of respective amino acid.Reaction mixture was maintained at 90 deg.C for one hr and then benzene was added to it slowly over a period of 5 mins.RM was now refluxed for 6-7 hrs.Water was removed from it using D&S apperatus.Complesion of reaction was checked by TLC.This provided compound 3 (a-e). Now in order to remove *p*-Toluene sulfonic acid,reaction mixture was extracted twice with methelene dichloride.The pH of RM was maintained with the help of liq. ammonia at 9.5±0.5 during first extraction and then to 8.5±0.5 during subsequent extractions.This provided the respective amino acid benzyl esters 4 (a-e) .

Next,the esters obtained in the previous step *were coupled with Valsartan via Dicyclohexyl carbodimide (DCC) coupling method, in order to obtain Valsartan intermediate 5(a-e). DCC coupling is one of the major tools employed in the literature to introduce peptide bonds by reaction of acids groups and amino acid esters[21].1-Hydroxy benzotriazole (HoBT) is widely used as an additive to decrease recimization in the carbodimide peptide coupling[22-24].To proceed for it,calculated amount of Valsartan (1mol)was dissolved in methelene dichloride and triethyl amine(2.8mol) at 18-20 deg.C. Temperature was further reduced to 10-15 deg.C.With continues stirring,calculated amount of HoBt(1.1mol) was charged suitably to the reaction mixture.Now,Benzyl ester of required amino acid (1mol) was charged,followed by addition of Dicyclohexylcabodimide (1.2mols).*

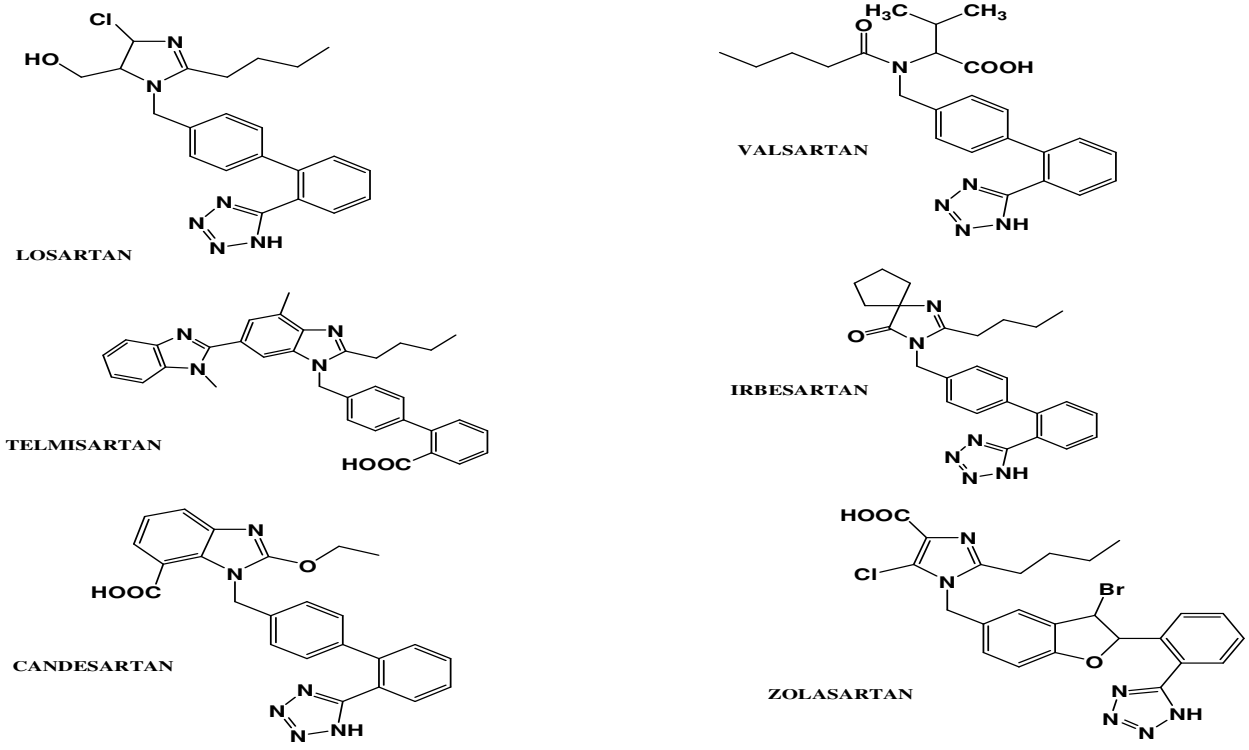
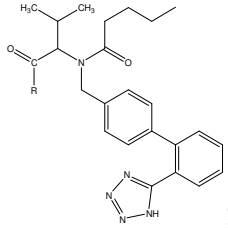
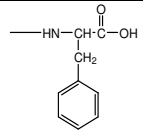
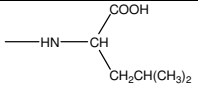
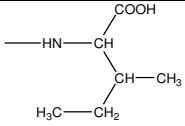
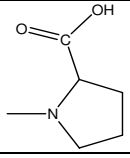
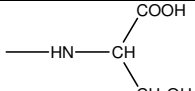


Figure 4. *Structures of various Angiotensin II receptor Antagonist*

TABLE No.2-synthesised analogues VH-6 to VH-10



1.valsartan

Comp. no2-5	a	b	c	d	e
entry	VH-6	VH-7	VH-8	VH-9	VH-10
R=					

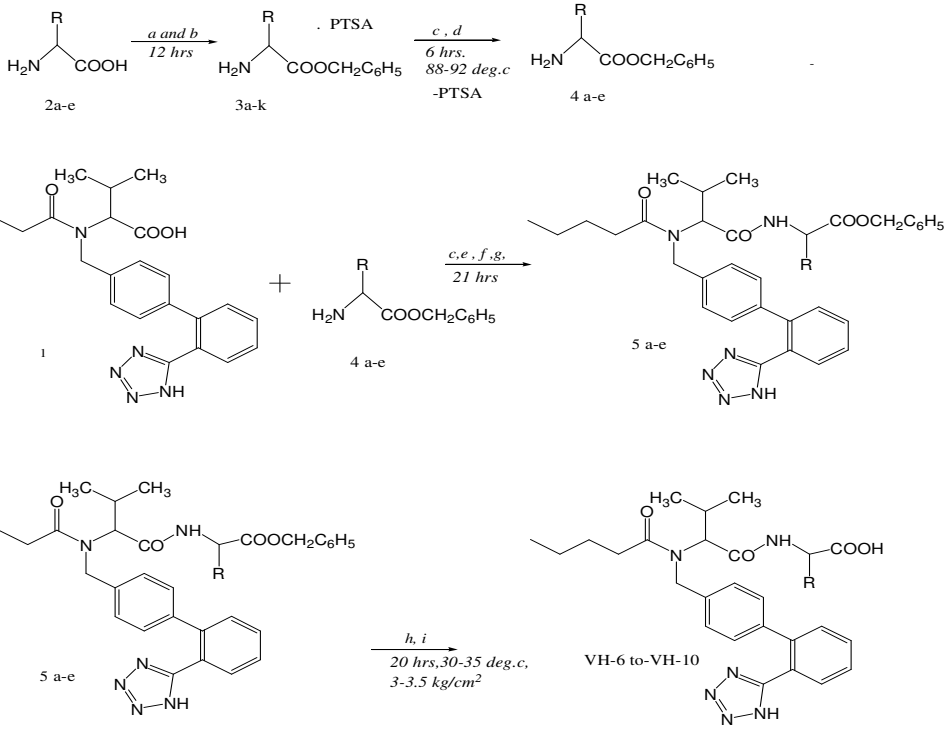


Figure 5. *Scheme of Synthesis-a- p-tolune sulphonic acid,b-Benzyl alcohol,c-Methelene Dichloride,d-Liq.ammonia,e-1-Hydroxy benzotriazole,f-Dicyclohexylcarbodimide,g-Triethyl amine,h-abs.Alcohal, i-5%Pd/C*

RM was brought to the room temperature and stirring was kept continued.Complesion of reaction was checked by TLC. Upon complesion, the reaction mixture was filtered ,MDC layer separated and distilled out to obtain valsartan intermediate compound 5(a-e).Final step was removal of benzyl group.This was accomplished through process of hydrogenation using 5% Pd/C at 30-35˚C and maintaining the pressure at 3-3.5 kg/cm^2,untill complesion of reaction.This provided us the required compounds VH-6 to VH-10[25]. **(See Fig. 4 and 5 & Table no. 2).**

The results are supported by suitable spectral data.NMR was done on Mercury plus300 MHz spectrophptometer,varian USA.Mass was done on 410 Prostar Binary LC with500 ms IT PDA detectors ,Varian USA .The instrument used for doing IR was MAGNA 550,Nicolet Inst. Corp.,USA.Elemental analysis was done at Center with Potential in Excellence in Biomedical Sciences, Chandigarh Punjab.

ADME PROPERTIES

ADME properties were calculated using Qikprop 2.5 tool of Schrodinger software.It predicts both physiochemically significant discriptors and pharmacokinetically relevant properties.Qikprop provides ranges for comparing particular property of a molecule,with those of 95% known drugs .Qikprop also flags 30 types reactive functional groups that may cause false positives in high throughput screening (HTS)assays.It also evaluates the acceptibilities of analogues based on Lipinski'srule of five, *[26,27]*which is essential to ensure their drug- like pharmacokinetic profile while doing rational drug design .In our present work,we analysed various descriptors and pharmaceutically relevant properties of the proposed compounds using Qikprop. Here we have reported some of the significant properties,required for predicting the drug like character of a molecule .These are :

1.Molecular Weight (mol_MW) (150-650)
2.Octanol/ water partition coefficient (Log Po/w)(-2 to 6.5)
3.Aquous Solubility (QP log S)(-6.5 to -0.5)
4.Blood/brain partition coefficient (QP log BB) (-3.0 to 1.2)
5.Persent human oral absorption (≥80 % is high, ≤25% is poor)

According to the Lipinski's rule of 5,for a candidate drug ,molecular weights should be less then 650,partition coefficient between octanol water(logPo/w)between -2 to 6.5 and solubility (QPlog S)greater then -6.5.All the proposed compounds were found to have significant values for the properties analysed and showed drug like charecteristics based on the rule .Blood/brain partition coefficient(QPlog BB)parameter indicates about the ability of of the drug to pass through the blood brain barrier.All designed compounds showed the ADME values in acceptable range .The values of selected prorperties are given in **Table no.-3.**

Table no.3 Prediction of ADME properties of Designed analogs using Qikprop:-

S.NO.	COMP.	MOL.WT	LOGP O/W	LOG S	LOG BB	ORAL ABS.*	RULE OF 5
1.	VH-6	582.70	4.485	-5.947	-2.77	58%	1
2.	VH-7	548.68	3.94	-5.858	- 2.944	54%	1
3.	VH-8	548.68	3.955	-5.729	-2.797	56%	1
4.	VH-9	532.64	3.043	-4.494	-1.881	59%	1
5.	VH-10	532.60	2.446	-4.523	-3.525	22%	2
6.	VALSARTAN	435.52	3.805	-4.774	-1.588	82%	0

*In case of humans

BIOLOGICAL EVALUATION

The newly synthesized compounds were further subjected to pharmacological evaluation.Their in-vivo anti-hypertensive activity was determined by Non Invasive method as well as by Invasive method of blood pressure measurement in rats.The

experimental protocol involving use of animals for the study was approved by the duly constituted Institutional animal ethical committee, Tumkur. Reg no. /CPCSEA dtd 19-5-1999 under the rule 5(a) of breeding and experiments on animals (control and supervision rules 1998) ;Ref:SSCPT/IAEC.Clear/102. The animal were taken care of, in accordance with the guidelines of committee, for purpose of control and supervision of experiments on animals (CPCSEA).

*In case of non invasive method,the blood pressure measurement was done in conscious rat by the tail cuff[28-30]. The 2K-1C Goldblatt model was used in order to induce renovascular hypertension in rats[31,32].The kidneys normally receive approximately one quarter of the cardiac output. Under normal physiological conditions, fluid and electrolyte homeostasis is maintained by alternations in renal perfusion and glomerular filtration. Goldblatt and coworkers first described the early biochemical abnormalities that occur after experimental clipping of the renal artery in dogs in 1934. Acute unilateral RAS (Goldblatt two-kidney, one-clip model)with a reduction in renal blood flow below a critical perfusion pressure of 70–80 mm Hg., activates stretch-sensitive receptors within the juxtaglomerular apparatus and sensors of sodium delivery to the macula densa. These receptors then stimulate the constitutive release of renin by the juxtaglomerular cells of the afferent arteriole.Our experiment was designed in accordance to above facts. M*ale Sprague Dawely rats weighing 180–200 g were anaesthetized by intra peritoneal injection of 60 mg/kg thiopental sodium, *and after a midline laparotomy,a silver clip with an internal diameter of 0.20 mm was placed around the left renal artery. The 2K rats were submitted to laparotomy only.*

Table no.4 - SBP RESPONSE WITH TIME FOR NON INVASIVE METHOD **:**

S.NO./test sample	base line(SBP in mm Hg)	After 30 min(SBP in mm Hg)	After 60 min(SBP in mm Hg)	After 120 min(SBP in mm Hg)	After 180 min(SBP in mm Hg)
1.VH-6	148	145	142	138	135
2.VH-7	152	146	144	140	137
3.VH-8	140	135	126	120	120
4.VH-9	153	144	141	140	136
5.VH-10	144	139	136	131	127
6.losartan	132	124	119	110	109

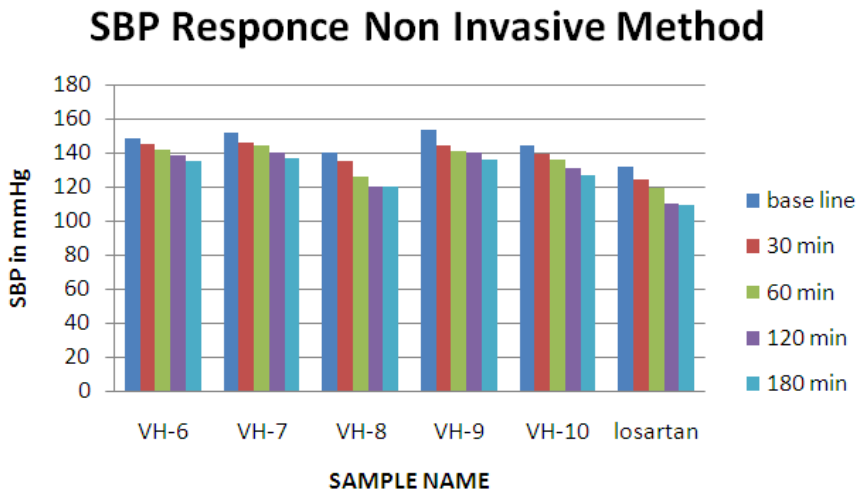


Figure 6: Non Invasive Method: Graphical representation of SBP Response

The rats were maintained on standard rat chow with a 12-h light/dark cycle and given free access to food and water. Five week after clipping, blood pressure was measured and rats with the value higher than 130 mm.Hg were selected for the experiment. All the rats were pre-conditioned to the experimental conditions before actual measurements were conducted.At the time of experiment the rats were placed in a constant temperature chamber at 32^{o} C for 30 min. Thereafter, the animals were put in a rat holder.and the drug was administered orally at dose level of 10 mg /kg animal body weight[33]. Systolic blood pressure (SBP)was measured in conscious rats by the tail cuff method . The tail cuff and pulse sensor was placed on the tail and connected to a rat-tail blood pressure monitor (Harvard Apparatus). The pressure in the cuff was displayed on a computer connected with blood pressure monitor (BIOPAC Systems, Inc. MP100A-CE Santa Barbara, California). SBP was measured at the point where the reappearance of pulsations was detected by the pulse sensor.For each rat, eight individual readings were obtained. The highest and the lowest measurements were rejected and average of remaining was taken as the individual SBP.After administing test compound to the rats SBP was measured at regular interwals ,over a period of 180 mins .The compounds undertest elicited a significant decrease in SBP. Losartan was taken as the std drug for comparision .Maximum lowering of elevated blood pressure was observed in case of comp.VH-8, where SBP was lowered by 20 mm Hg.,while it was lowered by 17 mm of Hg in case of both comp.VH-9 and VH-10.Figure 6 shows Graphical representation of SBP response by Non Invasive method. *(***See Table 4 & Fig. 6).**

The in vivo antihypertensive activity of the proposed compounds was determined by invasive method also in Ang II induced hypertensive rats.Instrument used was BIOPAC Systems, Inc. MP-36 Santa barbara California for recording blood-pressure response . Male albino rats, weighing 300-400 g were used.They were anesthetized with intraperitonial injection of urethane hydrochloride,1.25g/kg.They were further prepared by shaving the neck and inguinal region with animal hair clippers. The jugular vein was Surgically cannulated for drug administration.Left carotid artery was Isolated and exposed by dissection for blood pressure recording, using PE-50 tubing. The arterial cannula was Connected via the BSL pressure transducer (SS13L) to the BIOPAC Systems, Inc. Criterion for antihypertensive activity was considered as reduction of systolic arterial pressure by about 10-20 mmHg.

Angiotension-II- induced hypertension was obtained by administering Angiotension-II, 0.5 µg/kg i.v.It was then allowed to return to pre-injection level. In order to see the effect of antihypertensive compounds on induced hypertension, the test compound was next injected as 1 mg/kg solution i.v., and the system was allowed to equilibrate. Angiotension-II, 0.5 µg/kg i.v. was repeated as described previously.Finally blood-pressure response was observed and recorded for each rat[34,35].

TABLE 5: INVASIVE METHOD: SBP RESPONSE

Comp.Under Test	SBP in mm Hg. With ANG II Alone	SBP in mm Hg. With ANG II +TEST	Lowering of SBP
VH-6	136.68	128.23	-29.5
VH-7	155.99	127.58	-30.81
VH-8	118.88	104.26	-29.1
VH-9	158	130.26	-11.7
VH-10	146	114	-30.96
Valsartan	220.9	152.2	-78.7
Losartan	184.7	163.6	-21.1

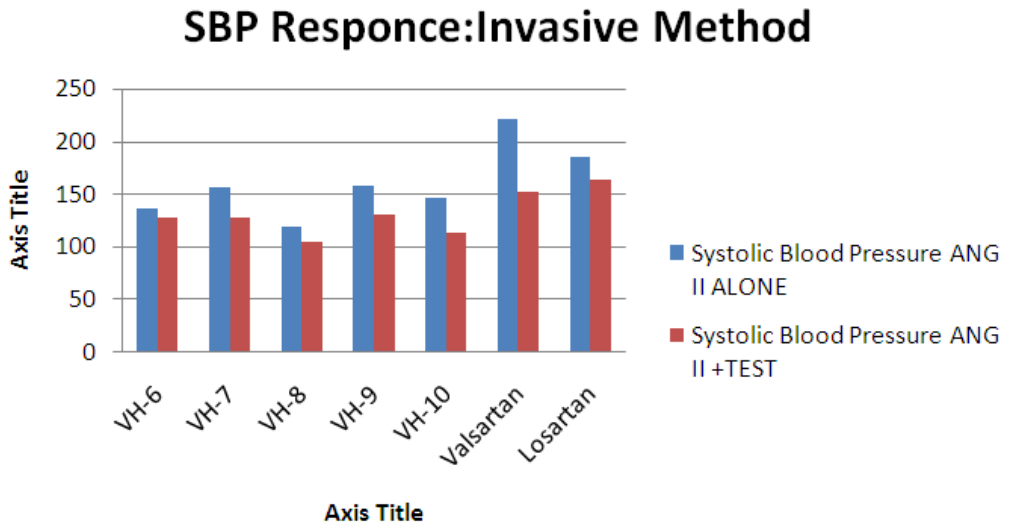


Figure 7. Invasive Method: Graphical representation of SBP Responce in case of 1)Ang II alone and 2)Ang II+Test

The sample alone in the dose of 01mg/kg i.v. did not have any effect in the baseline reading such as systolic blood pressure (SBP),diastolic blood pressure(DBP),mean arterial blood pressure (MABP) and heart rate (HR).Administration of Angiotension-II in the dose of 0.5 µg/kg i.v. significantly increased SBP, DBP, and MABP but did not modify HR. { [a]P<0.01 compared with base line readings}.When the samples were administered in the dose of 01mg /kg i.v. on Angiotension-II induced hypertensive rats, it was observed that SBP, DBP, and MABP were significantly reduced, whereas HR slightly increased. { [b] P<0.01 compared with Angiotension-II alone readings}.Maximum reduction of systolic blood pressure was found in case of VH-7 and VH-10 where it reduced to 31 mm Hg and 30.8 mmHg respectively. The reduction in case of VH-6 was 29.5 mm Hg,while in case of VH-8 and VH-9 it was found to be 29.1 and 11.7mm Hg respectively. Results of B.P.responces obtained by invasive method are shown graphically **(See table 5& Fig. 7).** (Values of MABP,SBP,DBP and HR for all compounds reported here are available with the author).Analysis of results obtained upon pharmacological evaluation clearly indicate that the Compounds VH-6 to VH-10 are having a fairly good anti-hypertensive activity.In particular compounds VH-6,7,VH-8 ,VH-10 are better in terms of efficacy.The antihypertensive effect may be due to decreasing the peripheral vascular resistance.

CONCLUSION

In summary a series of N-substituted amino acid derivatives as Angiotensin II receptor antagonist were designed and synthesized and were biologically evaluated for Antihypertensive activity.Homology modeling and Docking studies were also performed for the proposed derivatives using software schrodinger. Docking studies indicate that the proposed derivative show interactions with the recidues, corresponding to those reported earlier. The antihypertensive activity was determined both by Invasive as well as Non Invasive blood pressure measurement techniques. All the newly synthesized derivatives showed considerably good antihypertensive activity by invasive method as well as by non invasive method .Further, upon comparing efficacy tested by invasive method, VH-7and VH-10,amongst all derivatives were found to be most efficient in lowering the elevated blood pressure.Similarly according to the results of non invasive method VH-7 gave maximum lowering of elevated blood pressure.Along with this VH-10 also gave fairly good results by non invasive method

which are comparable to the standerd compounds . Good activity may be assigned to *the fact that nitrogen containing building blocks play an important role in drug design and provide enhanced interaction between pharmacophore and receptor sites.Another important fact for good activity is that amino acid recidues can often interact with the active site of receptors and play a pivotal role via H -bond and charge effect.The* antihypertensive activity was found dependent upon the overall lippophilicity of the molecule too.All above facts *indicate that these compounds with structural novelty and excellent pharmacological properties can be considered as novel anti-hypertensive candidates and deserve further investigations.*

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