SUMMARY

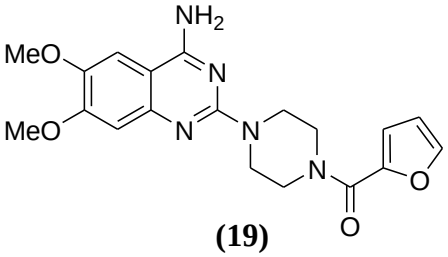
A report revealed, around 51% deaths worldwide are caused only due to cardiovascular diseases among the noncommunicable types of diseases such as respiratory disorders, cancer and diabetes. Since the pathophysiology of hypertension and related disorders is much complicated so there is a need to control them more skillfully. Despite the availability of a number of drugs no single drug can effectively control the raised blood pressure, so a major part of hypertensive population depends on the combination therapy to regulate the elevated blood pressure. The general targets involved in the treatment of hypertension and other cardiovascular diseases, are kidney, adrenergic receptors and blood vessels.

A need was felt for the development of drugs that have the potential to act on multiple receptors synergistically with improved patient compliance. Designing aspects, for the development of such a molecule that has the ability to work synergistically through different receptors are too complex in terms of metabolic stability and binding efficiency. Since the rationale, to design such type of molecules are mainly based on the fusion of the pharmacophoric features from different molecules, there are chances that such a new molecule would possess a higher molecular weight which generally would not fit in the pharmacokinetic features of the body, hence the binding efficiency of these molecules on the targeted receptors might be poor. Thus, there are challenges for the attainment of balanced activities on the projected targets with high selectivity and improved pharmacokinetics in the designing of such multitargated drugs.

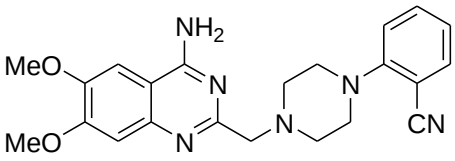
The α_1- and AT_1-receptors working through SNS and RAS, respectively have been proved as the prime targets to set the elevated blood pressure. Both these receptors could be targeted simultaneously for better control of blood pressure. Designing of multiple target-directed ligands is basically a challenging task in order to attain balanced activity with higher selectivity and admissible pharmacokinetic profile to the desired targets. To design such type of molecules it is requisite to know about the role of pharmacophores in prototype compounds.

Knowledge-based approach was used for designing of dual α_1 and AT_1 antagonists. This approach is based on combining of frameworks and underlying pharmacophores of two drug molecules, each selective for one particular target of interest, into a single chemical entity possessing both of the activities of the parent molecules. The resulting dual acting ligands could have linked, fused or merged pharmacophores. After the study of literature, it was envisaged to fuse the selected pharmacophores of two compounds *i.e.* prazosin (acting on α_1-receptors) and losartan (acting on AT_1-receptors) into a single chemical entity to afford balanced activity on the desired targets. The key points about both (α_1 and AT_1) the receptors have been considered from the literature.

Losartan (**13**) and prazosin (**19**) are the AT_1- and α_1-receptors antagonists respectively. Earlier studies revealed that the integration of pharmacophoric features of the α_1- and AT_1- selective molecules resulted into a new potent and dual acting chemical entity (**20**) as potent antihypertensive agent with ability to bind on both the receptors synergistically. The designed compounds were synthesized and evaluated for their biological activities on both the earlier discussed receptors. In the *in vitro* evaluation some of these compounds were found as potentially active on both the discussed receptors.[89]

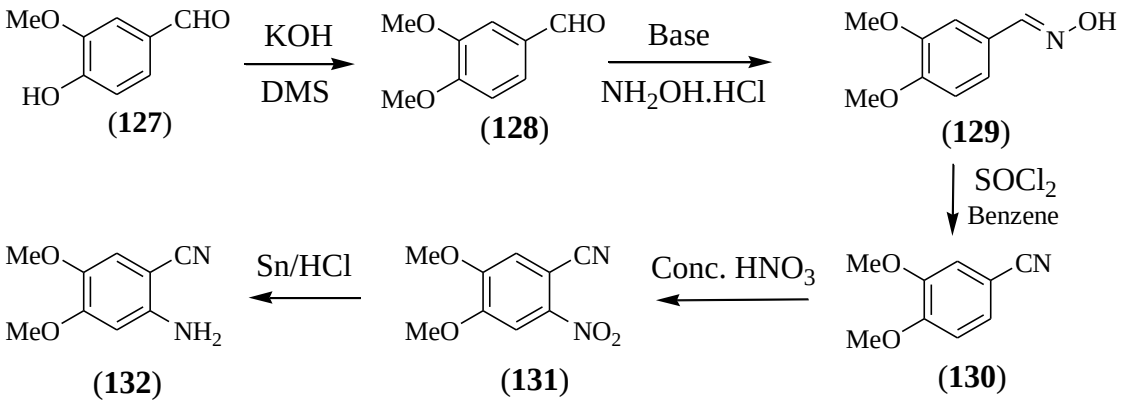


(19)



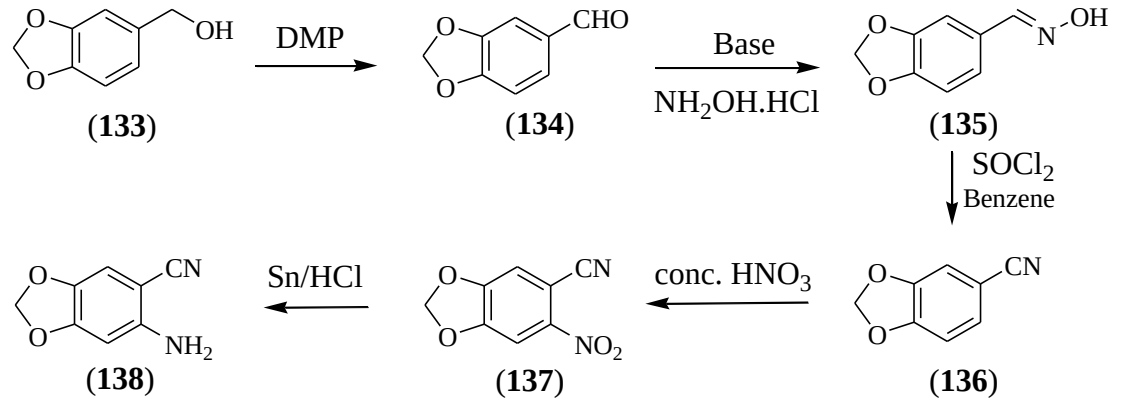
(20)

These findings created an interest to work further in this direction. Encouraged by the results, it was planned to synthesize compounds which could have better aqueous solubility and more structural variations. The intermediates and scaffolds required for the synthesis of the desired compounds were prepared in the following ways (**Scheme 1 to Scheme 6**):



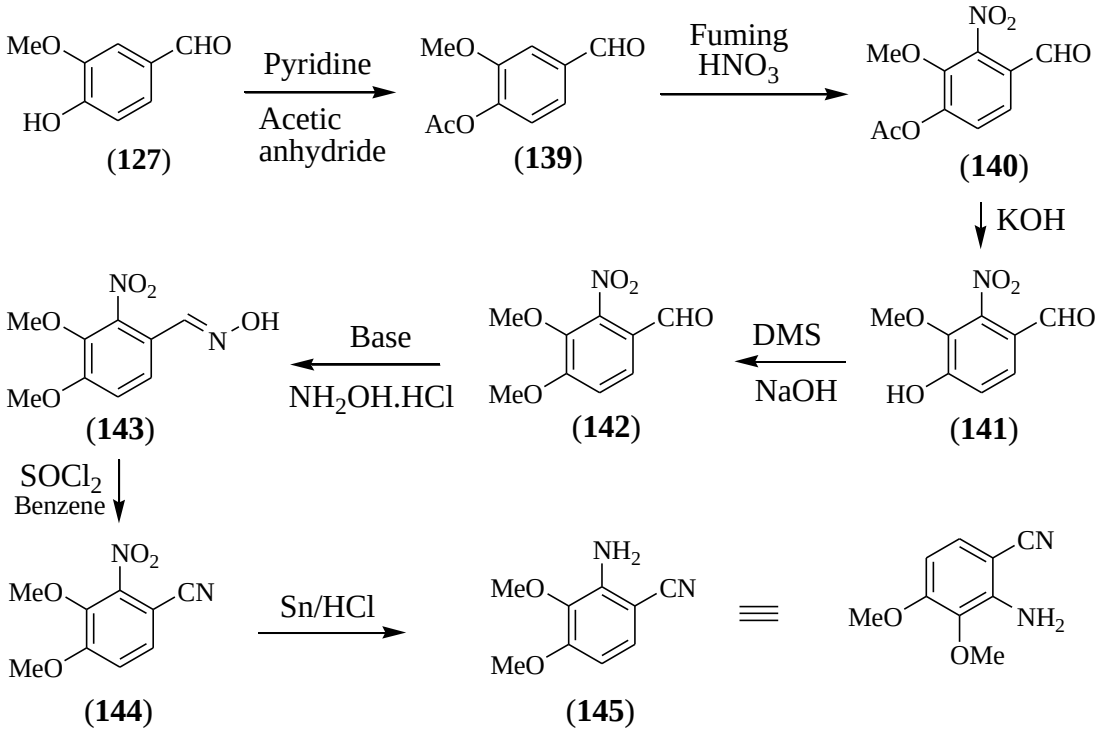
(**127**) (**128**) (**129**) (**130**) (**131**) (**132**)

Scheme 1: Synthesis of 2-amino-4,5-dimeyhoxybenzonitrile (**132**)

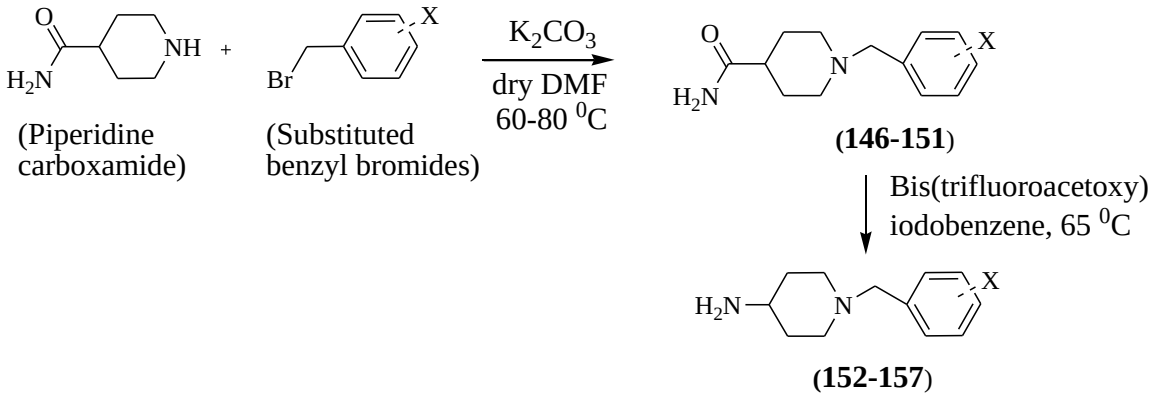


(**133**) (**134**) (**135**) (**136**) (**137**) (**138**)

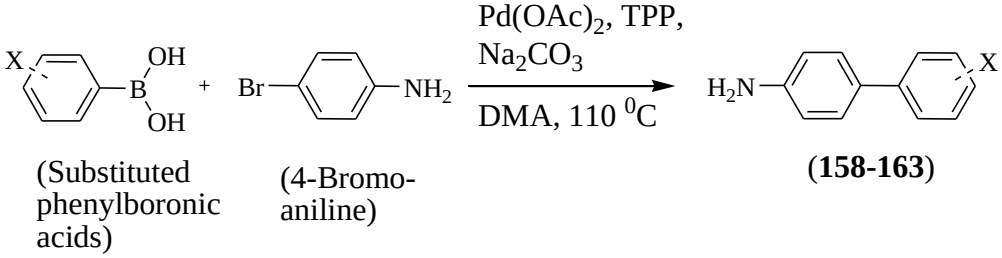
Scheme 2: Synthesis of 2-amino-4,5-methylenedioxybenzonitrile



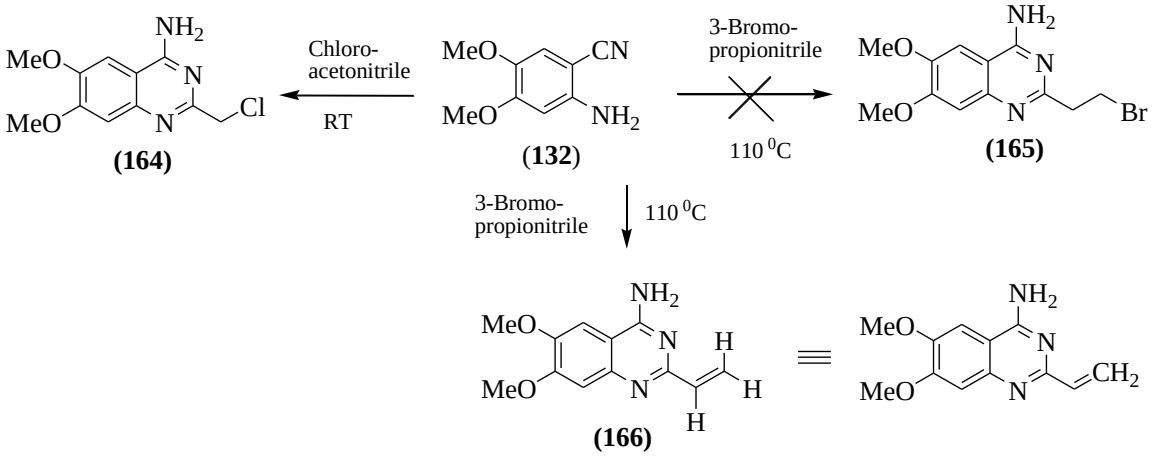
Scheme 3: Synthesis of 2-amino-3,4-dimethoxybenzonitrile



Scheme 4: Synthesis of 2'/4'-substituted benzylpiperidin-4-amines (**152-157**)

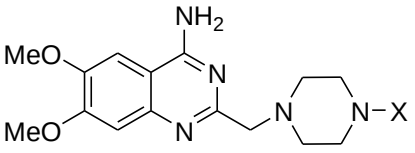


Scheme 5: Synthesis of substituted 4-aminobiphenyls (**158-163**)

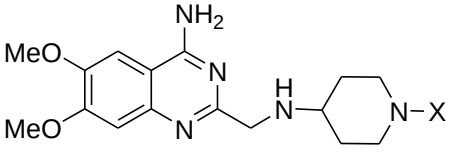


Scheme 6: Synthesis 4-amino-2-choloromethyl-6,7-dimethoxyquinazoline (**164**) and 4-amino-6,7-dimethoxy-2-vinylquinazoline (**166**)

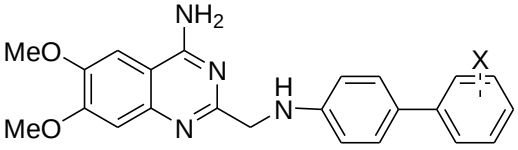
Using the above intermediates and scaffolds, the following seven series of compounds were aimed to be synthesized and their biological activities evaluated:



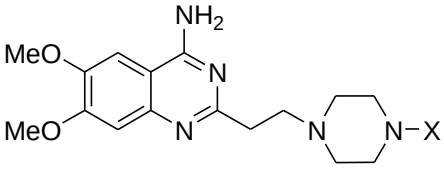
Series I (**167-192**)



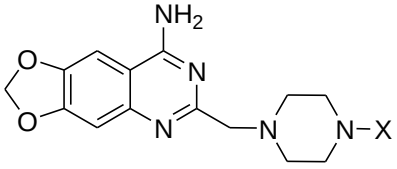
Series II (**193-198**)



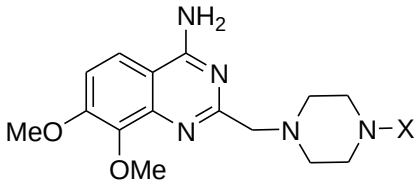
Series III (**199-204**)



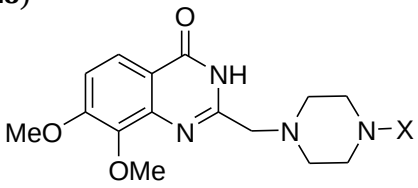
Series IV (**205-210**)



Series V (**212-228**)



Series VI (**230-242**)



Series VII (**245-251**)

All of the synthesized compounds were characterized on the basis of their spectral data. Methods of synthesis for these new compounds, their spectral (IR, ^{1}H-NMR, ^{13}C-NMR and Mass spectrometry) data and biological activities have been discussed in detail in the thesis.

The synthesized compounds were screened for antagonistic activity on both α_1- and AT_1-receptors separately by *in vitro* screening on isolated tissue preparation (Table 6). Compounds (**167-192**) having a methylene spacer between the 4-amino-6,7-dimethoxyquinazoline moiety and the piperazine ring and a keto spacer (-C=O) between the piperazine ring and the phenyl ring (**165-174**) yielded compounds with variable potencies. Compound (**176**) showed balanced but poor activity (pA_2 for α_1 = 6.93 and AT_1 = 6.87) on both the receptors. Compounds (**167-169**) were also found to be poor to moderately (pA_2 = 4-7) active on both the receptors but the activities were not balanced. Compounds (**172** & **173**) having dichloro and dimethoxy groups at 2nd and 3rd positions of the phenyl ring were observed to be moderately active at AT_1-receptor but inactive at α_1-receptor. Compounds (**170** & **174**) were found to be inactive on both the receptors. Compounds (**177-185**) in which phenylpiperazinylmethanone moiety was reduced into benzylpiperazine, offered better results in the preliminary screening. Compound (**179**) (pA_2 for α_1 = 8.77 and AT_1 = 8.60) among them, showed almost equal antagonistic potency to the standard drugs terazosin and losartan. Compounds (**177**, **182**, **183** & **185**) also showed dual antagonism but the potencies were ranging from poor to moderate. Compound (**178**) having strong electron withdrawing group (CF_3) at 2nd position of the phenyl ring was found to be moderately active at AT_1-receptor but inactive at α_1-receptor. Compounds (**180, 181**, **184** & **186**) were found to be moderately active at α_1-receptor but were inactive at AT_1-receptors. Compound (**188**) having benzhydryl moiety showed balanced antagonistic activity at both the receptors but showed lower potencies than terazosin for α_1- and losartan for AT_1-receptor. Compound (**187**) having 1-naphthyl moiety showed similar type of results as shown by compound (**188**).

On the other hand conversion of phenylpiperazinylmethanone moiety into 2-phenylpiperazinylethanone as in compounds (**190-192**), failed to produce satisfactory results on both the receptors. Although compound (**191**) showed balanced activity but it

was no where comparable to the standard drugs (terazosin and losartan). Compound (**192**) was found to be inactive on both the receptors.

Compound (1**99**) showed somewhat similar (pA_2 for α_1 = 7.82 and AT_1 = 7.99) potency to compound (**179**). Compounds (**200**-**204**, **214**-**218**, **221**, **224**, **225**, **228** & **230**) displayed activities in the pA_2 range of 4-8. Compounds (**220** & **226**) offered moderate activities only for α_1-receptor.

Thus, compound (**179**) amongst all of the test compounds of the series was found to be the most potent dual antagonists in the *in vitro* tissue experiments. As shown in Figure 5, compound (**179**) showed competitive antagonism against phenylephrine and angiotensin II. A rightward parallel shift was observed in the dose response curve after addition of compound (**179**). The results demonstrated competitive dual antagonism by compound (**179**) on α_1- as well as AT_1-receptors.

Table 6: *In vitro* activities (pA_2 values for α_1- and AT_1-receptors) of the synthesized compounds

Comp.	(pA_2 Values)		Comp.	(pA_2 Values)		Comp.	(pA_2 Values)	
	α_1	**AT_1**		**α_1**	**AT_1**		**α_1**	**AT_1**
167	5.95	4.75	**187**	6.32	6.95	**207**	9.29	ND
168	5.66	7.75	**188**	**7.44**	**7.26**	**208**	9.27	ND
169	6.46	5.37	**189**	*	6.23	**210**	6.81	8.62
170	*	*	**190**	1.98	3.03	**213**	6.16	*
171	2.14	4.36	**191**	4.05	5.41	**214**	8.33	5.80
172	*	6.61	**192**	*	*	**215**	6.37	4.70
173	*	6.34	**193**	4.95	5.51	**217**	6.07	5.96
174	*	*	**194**	6.43	ND	**218**	6.06	5.42
175	3.28	2.15	**195**	6.55	5.78	**220**	5.68	*
176	6.93	6.87	**196**	6.79	5.66	**221**	7.57	5.25
177	4.55	5.22	**197**	6.75	5.96	**224**	6.14	6.07
178	*	6.10	**198**	6.79	6.52	**225**	6.23	6.21
179	**8.77**	**8.60**	**199**	**7.82**	**7.99**	**226**	5.06	*
180	5.08	*	**200**	6.39	7.20	**228**	6.69	5.66
181	5.89	*	**201**	6.92	6.90	**230**	6.42	4.46
182	5.91	6.49	**202**	6.37	6.92	**Terazosin**	8.51	6.24
183	5.91	6.12	**203**	6.10	7.71	**Losartan**	5.46	8.08
184	7.19	*	**204**	6.32	5.31	**20**	**10.10**	**8.83**
185	7.50	6.58	**205**	9.24	ND			
186	4.64	*	**206**	8.81	ND			

*Did not show any activity
ND = activity not determined

Preliminary screening results showed that compound (**179**) possessed promising balanced antagonistic activity on both of the stated receptors (pA_2 for α_1 = 8.77 and AT_1 = 8.60). So, it was planned to revalidate the *in vitro* results into *in vivo* animal model for compound (**179**). Since invasive blood pressure (IBP) measurement provides direct assessment of antihypertensive activity of an investigational compound, it has been utilized as a gold standard technique for evaluation of potential antihypertensive compounds. Compound (**179**) was evaluated against phenylephrine (6 µg/kg) and AII (6 µg/kg) induced rise in BP in the rat model. In earlier findings, it has been proved that fall in blood pressure mediated by prazosin was not only because of α_1 antagonism (pA_2 for α_1 = 8.91), but also due to simultaneous blockade of AT_1-receptor (pA_2 for AT_1 = 8.26). Thus, for the present study terazosin was chosen as a standard α_1 antagonist instead of prazosin and losartan as a standard AT_1-receptor antagonist against phenylephrine and AII mediated pressor responses respectively.

For the purpose of comparison, the dose levels of the agonists and antagonists were maintained as per the previous study. Despite having almost equal or higher potency then terazosin in the *in vitro* experiments, the selected compound (**179**) could not replicate the response of the standard α_1-blocker terazosin in the *in vivo* animal model. This is probably due to the distribution of the test compound (**179**) on both the receptors, as it is dual antagonist while the standard terazosin inhibits only the α_1-receptor. So the experiments were repeated by masking one receptor at a time and evaluating the test compound on the other receptor. Terazosin (0.72 µM) was used for masking of α_1-receptor and the test compound (**179**) was evaluated for its antagonism of AII response while losartan (3.6 µM) was used for masking of AT_1-receptor and the test compound evaluated for its antagonism of phenylephrine response on α_1-receptor, as described in the previous report.

Under masking conditions, test compound (**179**) was found to be almost equipotent to the two standard drugs, terazosin as α_1-antagonist and losartan as AT_1-antagonist supporting the observations of the *in vitro* experiments of dual antagonist nature of the synthesized compound.