2. Literature review

2.1 AT_1- and α_1-receptors and their antagonism

Peripherally increased vascular resistance imbalances body hemodynamic system which results into high blood pressure.[90] Sympathetic nervous system (SNS) and renin-angiotensin-aldosterone system (RAAS) are the two important contributing systems to regulate the vascular tone. These two systems are affected by mutual interactions and mainly control the cardiovascular regulatory functions[7,91] as shown in **Fig. 4**. Positive inotropic and chronotropic effects are the results of the SNS stimulation while AII formation takes place due to activation of RAAS. AII increases systemic vascular tone through strong vasoconstrictory action that influences blood pressure with the release of aldosterone and noradrenaline. When body haemodynamics is imbalanced due to the

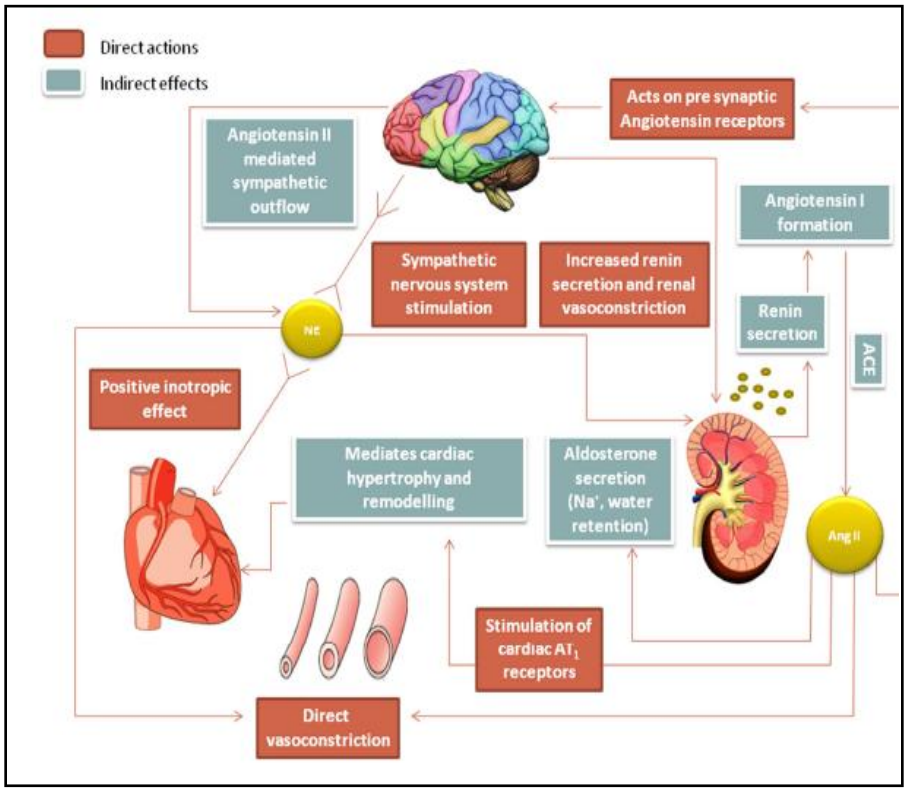


Fig. 4: Mutual coordination between SNS and RAAS

reduced renal blood flow (RBF), glomerular filtration rate (GFR) or urinary sodium excretion, the α-adrenergic receptors play homeostatic role by means of tubular gluconeogenesis and renal vasoconstriction which are coupled to tubular Na^+

reabsorption. The primary role of AII is retention of water and salt reabsorption by reducing the blood flow through the kidneys which resulted in vasoconstriction.[92]

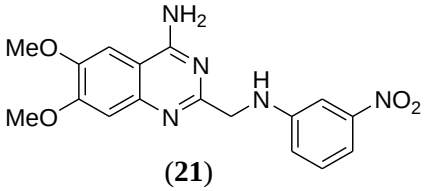
Following are the evidences that prove the mutual interactions between both SNS and RAAS:

- The secretion of renin is followed by the production of AII due to the influence of SNS.
- There occurs negative regulation of AII receptors in cultured brain neurons[93] and in vascular tissue under the influence of norepinephrine.[94]
- Intracerebrally administered AII raises blood pressure associated with systemic vasoconstriction which suggests a central facilitating effect of AII on sympathetic outflow. [92,95,96]
- In the neuroadrenergic transmission across sympathetic ganglia, AII plays an important role.[87,96,97]
- Under the influence of presynaptic angiotensinergic receptors, AII potentiates the release of norepinephrine.
- AII exerts a negative effect through baroreceptor reflex control on heart rate and sympathetic nerve conduction.[87,96]

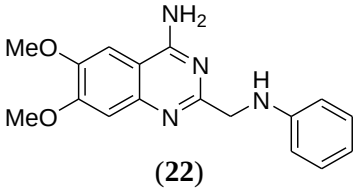
The importance of renin-angiotensin-sympathetic interactions on physiological and pathophysiological conditions is observed in cardiovascular disorders.[87,96,97] On the basis of the above discussion it becomes cleared that both SNS and RAAS play vital roles in the regulation of the blood pressure. Thus the current study involving synergistic antagonism of both the systems would be more advantageous than targeting an individual system. α_1- and AT_1-receptors are the two vital constituents of SNS and RAAS which work in unison in the regulation of blood pressure.

On the basis of SAR studies of AII-antagonists and prazosin (**19**)-type α_1-blockers, Yadav *et al*. designed and synthesized several molecules which antagonized both α_1 and AT_1 receptors simultaneously. Compounds (**20-22**), among them showed balanced activity on both the receptors in which compound (**20**) was found to be the most

potent in the *in vitro* evaluation. However, it was found to be slightly less potent than prazosin (**19**) but equipotent to losartan in the *in vivo* studies.[89]



(**21**)



(**22**)

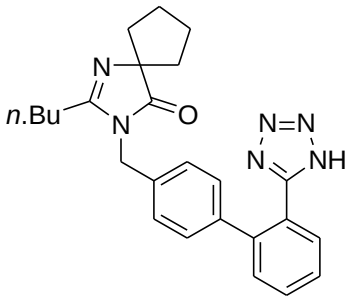
Comp.	*p*A_2 Values	
	AT_1-receptor	α_1-receptor
13	8.43	5.46
19*	8.26	8.08
20	**8.83**	**10.10**
21	9.09	8.09
22	8.37	9.87

*** Prazosin**

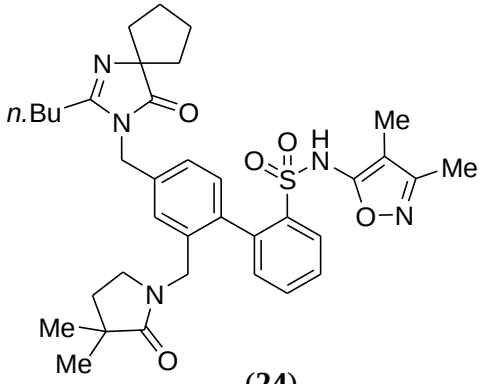
2.2 Dual acting agents used in cardiovascular disorders

2.2.1 AT_1- and ET_A-receptor antagonists

Murugesan *et al*. designed a series of sulfonamide derivatives such as BMS-193884 (**16**) by merging the structural features of typical AT_1- and ET_A-receptor antagonists. They maintained the biphenyl moiety in the newly synthesized derivatives, as it was a common structural feature for AT_1-receptor antagonism e.g. in irbesartan (**23**).



(**23**)

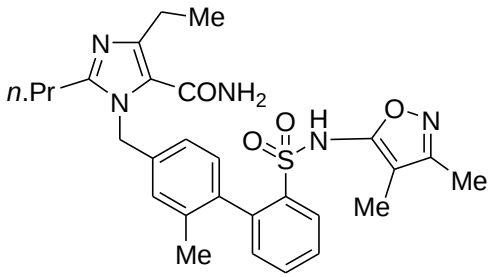


(**24**)

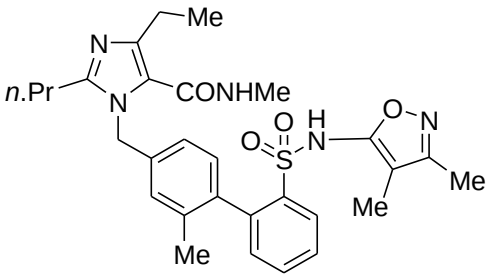
Comp.	K_i (nM)	
	ET_A-receptor	AT_1-receptor
16	1.4	$> 10^3$
23	$> 10^3$	0.8
24	1.9	10

The resulting compounds were evaluated for antagonistic activities on both AT_1- and ET_A-receptors. Compound (**24**) (BMS-248360) among them emerged as a potent dual antagonist for AT_1- and ET_A-receptors with improved oral bioavailability.[75]

Tellew *et al*. designed a series of 4-[(imidazol-1-yl)methyl]biphenylsulfonamides in order to obtain potent antagonists active on both the (AT_1 and ET_A) receptors. Some of these derivatives were orally active with improved antagonistic activity. Their designing strategy was based upon the fusion of structural elements of losartan (**13**) and BMS-193884 (**16**). Compound (**25**) among the synthesized derivatives was found to be the most potent in the *in vitro* evaluation. Compounds (**25** and **26**) proved to be orally active in the AII induced rat model.[98]



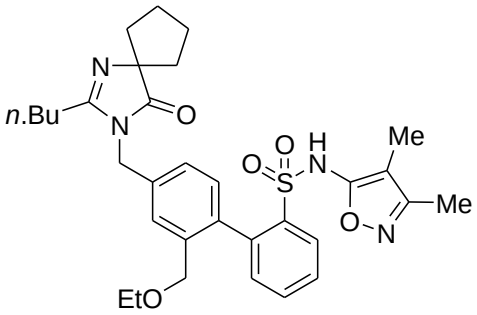
(**25**)



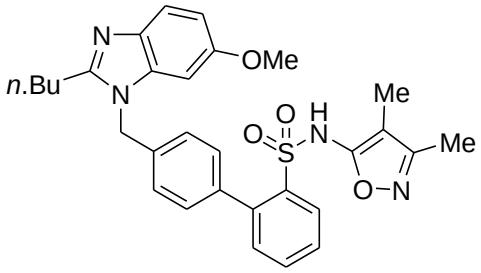
(**26**)

Comp.	K_i (nM)	
	AT_1-receptor	**ET_A-receptor**
25	2	5
26	4	5

Murugesan *et al*. made further efforts on their previously synthesized dual acting compound (**24**) in order to improve the pharmacokinetic features and potency for both the (AT_1- and ET_A-) receptors. In this direction they designed and synthesized some newer derivatives of compound (**24**). Their efforts were focused on the modification of the side chain at the 2'-position and of isoxazolylsulfonamide moiety in **24**. These efforts yielded a highly potent dual acting compound (**27**) which blocked efficiently both the (AT_1; Ki = 0.8 nM and ET_A; Ki = 9.3 nM) receptors. It also showed an improved pharmacokinetic profile over compound (**24**). Additionally, compound (**27**) efficiently reduced the AII and ET-1 induced blood pressure for a longer duration than **24**.[99]



(**27**)

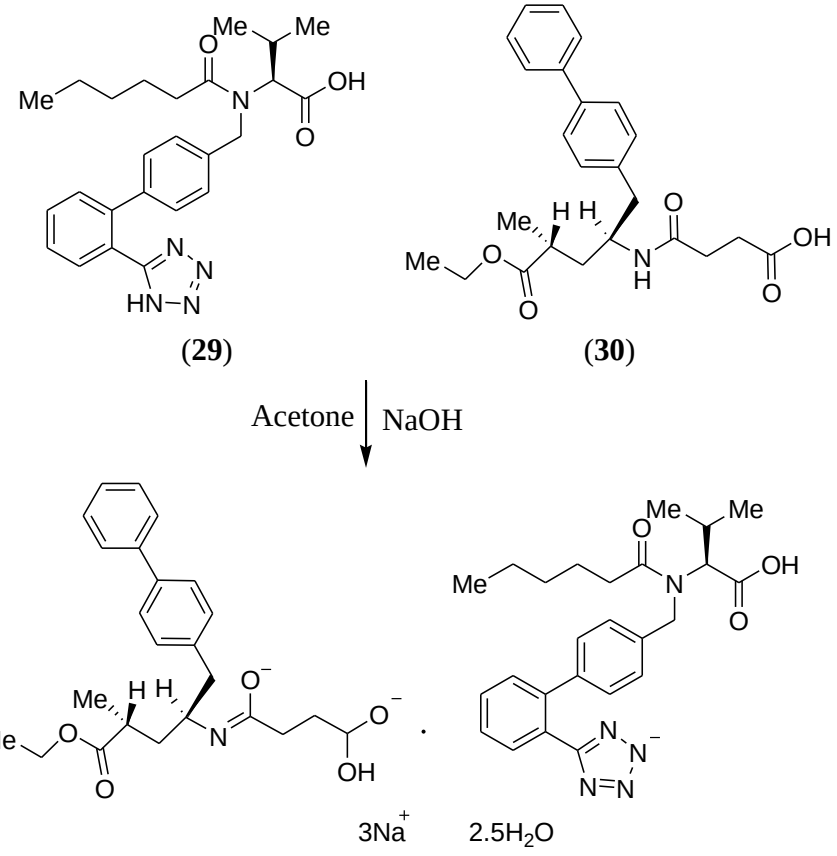


(**28**)

Bai *et al*. designed and synthesized a series of 4'-[(benzimidazol-1-yl)methyl]biphenyl-2-sulfonamide derivatives. One (**28**) of the compounds from this series was found to be the most potent antagonist for both the receptors (AT_1; IC_{50} = 8.5, ET_A; IC_{50} = 8.9 nM), without affecting the heart rate.[100]

2.2.2 Combined targeting for AT_1-receptor and NEP

Feng *et al*. prepared a unique complex molecule LCZ696 (**31**) which was compri-

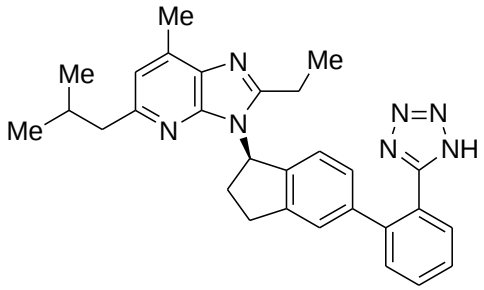


(**31**)

sed of 6 molecules of valsartan (**29**) and 6 of NEP inhibitor (AHU377) (**30**) in their anionic forms, 18 Na^+ ions and 15 molecules of water. Surprisingly, it was stable as a supramolecular complex with multi-coordinated sodium ions and water molecules. It showed AT_1 blockade with simultaneous inhibition of NEP. Recently it was developed by Novartis laboratories, as a drug and approved by US FDA. Chemically, Na^+ ions were connected with oxygen ligands derived from carboxyl and carbonyl functionalities of valsartan (**29**) and AHU377 (**30**), through coordinate bonding with entrapped water molecules. The basis of stability for this supramolecular complex was mainly a network of hydrogen bonds.[101]

2.2.3 AT_1-Antagonism along with PPAR-γ activation

Agustin *et al*. reported a series of imidazo[4,5-*b*]pyridines which showed dual activities on AT_1-receptors and peroxisome proliferator activated receptor-γ (PPAR-γ). The availability of the crystal structure of the lead molecule (**18**) led to design of a new scaffold having the affinity to bind both PPAR-γ and AT_1-receptors. The mode of action of this scaffold on the PPAR-γ was proved on the basis of available crystal structure of **18**. The designed scaffold gave the most potent AT_1-receptor antagonist (*S*)-3-(5-(2-(1*H*-tetrazol-5-yl)phenyl)-2,3-dihydro-1*H*-inden-1-yl)-2-ethyl-5-isobutyl-7-methyl-3*H*-imida-

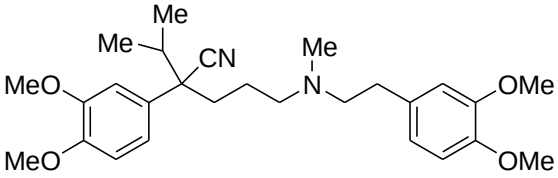


(**32**)

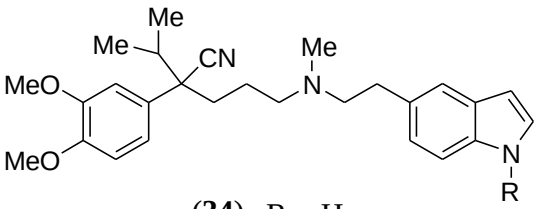
zo[4,5-*b*]pyridine (**32**) (IC_{50} = 1.6 nM) which was also reported to have partial PPAR-γ agonistic activity (EC_{50} = 212 nM) with improved oral bioavailability. The dual activity of **32** was biologically evaluated in hypertensive and insulin resistant animal models. Compound (**32**) potentially reduced blood pressure and lowered the glucose and triglycerides levels simultaneously.[102]

2.2.4 Dual antagonism through α_1-receptor/calcium channel blockers

Soll *et al*. synthesized some indole congeners of verapamil (**33**) on the basis of bioisosterism between catechol (a phenol) and indole-amines, and biologically evaluated them for calcium entry blockade into the ion channels with simultaneous antagonism of α_1-receptors. Among them, the indole congeners (**34** and **35**) emerged as dual blockers for α_1-receptors and calcium channels in the *in vitro* testing. Although in the *in vivo* studies compound (**34**) was found to be less active than verapamil (**33**) as an antihypertensive agent.[103]



(**33**)



(**34**) R = H

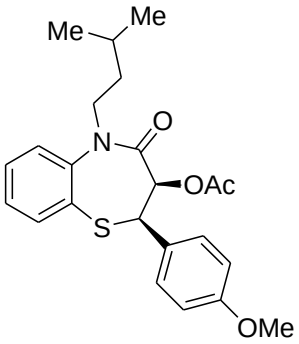
(**35**) R = Me

Comp.	Blockade of calcium entry (-log IC_{50})	α_1-Receptor antagonism (-log K_b)
33	6.5	5.8
34	6.5	7.5
35	6.6	6.1

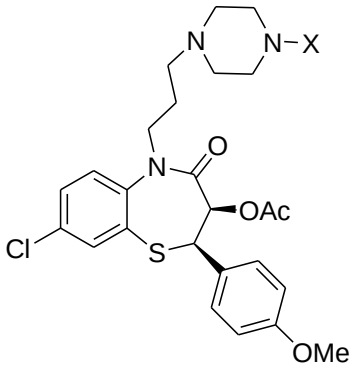
Yamamori *et al*. reported some derivatives (**37-42**) of 1,5-benzothiazepine as calcium channel blockers with additional coronary vasodilatory activity having the potency to reduce blood pressure and heart rate simultaneously comparable to diltiazem (**36**).[104]

Masui *et al*. studied the pharmacodynamics of S-2150 (**38**) using [^{3}H]diltiazem (**36**) and [^{3}H]WB4101 (**43**). Its biological effects were evaluated on coronary occlusion induced myocardial infarcted lesions and compared them against prazosin (**19**) and diltiazem (**36**). S-2150 (**38**) was found as effective in the reduction of size of myocardial infarcted lesions as diltiazem (**36**) whereas prazosin (**19**) was ineffective. They also compared the direct effects of **36** and **38** on coronary perfusion pressure (CPP) and cardiac functions, using isolated heart of rat. Both the drugs were able to increase the

coronary flow and to reduce the mechanical functions. Additionally, they proved that S-2150 (**38**) was more cardioprotective than **36** due to its controlled vasodilatory activity. These combined activities of S-2150 (**38**) could be explained by its dual blockade of calcium channels and the α_1-receptors.[105]

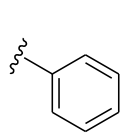


(**36**)

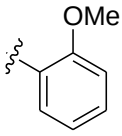


(**37-42**)

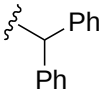
X =



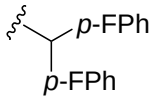
(**37**)



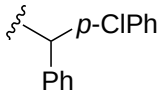
(**38**)



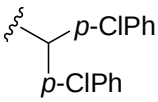
(**39**)



(**40**)



(**41**)

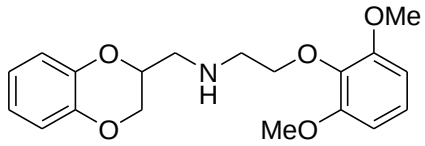


(**42**)

Comp.	Decrease in blood pressure (%)	Decrease in heart rate (%)
36	11	15
39	16	12
40	17	6.5
41	19	15
Comp.	**Calcium channels K_i (µM)**	**α_1-receptor K_i (µM)**
19	N.D.	0.001
36	0.120	>44
38	0.200	0.021

Toru *et al.*[106] studied the *in vivo* pharmacokinetics and pharmacodynamics of S-2150 (**38**) by intravenous infusions in the rat model using a combination of two sigmoid

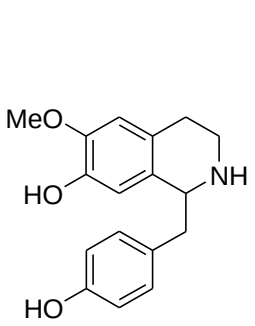
E_{max} models which were connected independently with the 'central compartment' and 'effect compartment'. Results of these kinetic studies supported the dual antagonistic activities of S-2150 (**38**).[106-108]



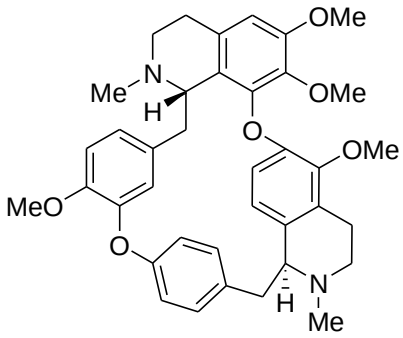
(**43**)

Iturriaga-Vasquez *et al*. synthesized some *O*- and *N*-substituted derivatives of (±)-coclaurine (**44**) having structural similarities with an alkaloid tetrandrine (**45**), in order to mimic its antihypertensive effects. All the derivatives were studied for their binding affinities to α_1-receptors and benzothiazepine modulatory site of the L-type calcium channels through radiolabeled ligand binding assays in cortical homogenates of rat brain, using radiolabeled [^{3}H]prazosin and [^{3}H]diltiazem. Some of them were selected for functional antagonism assay on both these targets using rat aortic strips which were pre-contracted with noradrenaline or depolarized with potassium chloride (KCl), respectively. Among them, compounds (**46** & **47)** were comparable with coclaurine and tetrandrine. It was proved that insertion of *O*-benzyl moiety in coclaurine (**44**), led to the appearance of calcium channel blocking activity along with α_1-antagonism in the same concentration range. These *O*-benzyl derivatives (**48-51**) of coclaurine were found much more effective than tetrandrine in reducing the contraction of aortal rings caused by noradrenaline or KCl. Improved binding affinities of *O*-benzylated derivatives (**48-51**) suggested that the large lipophilic moieties at 7th or 12th positions of the BTHIQ skeleton favor binding at benzothiazepine modulatory site. The binding affinities of **44** and its *O*-methylated derivatives (**52** & **53**) for α_1-receptors decreased with increasing methylation, suggesting that, at least one hydroxyl group was required for binding to the prazosin sites. While, a benzyl ether substituent was favorable for binding on these sites even on methylation of the other hydroxyl group, which suggested that the additional benzyl group might be forming hydrophobic interactions with relatively distant moieties, to compensate for the loss of hydrogen bond donating hydroxyl groups. In conclusion, cumulatively increased concentrations (10^{-9}-10^{-4} M) of these compounds inhibited both noradrenaline and KCl

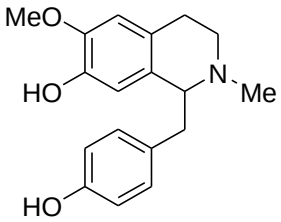
induced contractions, confirming their simultaneous antagonism of the adrenoceptors and calcium channels both.[109]



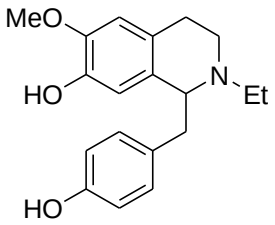
(**44**)



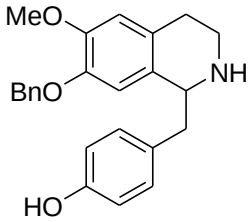
(**45**)



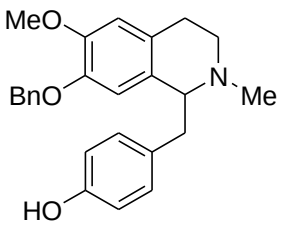
(**46**)



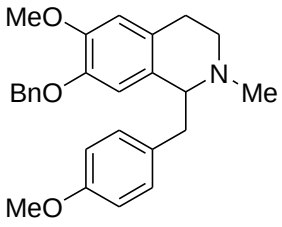
(**47**)



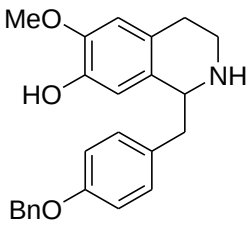
(**48**)



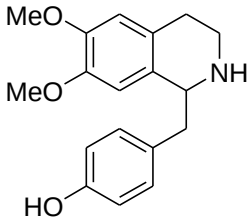
(**49**)



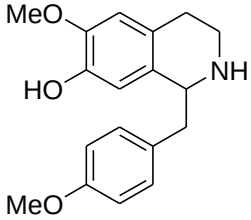
(**50**)



(**51**)



(**52**)



(**53**)

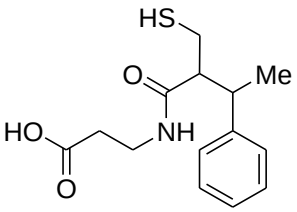
Comp.	Inhibitory potencies (pIC_{50})	
	Noradrenaline (NA) (1 µM)	KCl (80 mM)
44	4.76	ND
45	4.53	4.87
46	4.61	4.17
48	4.88	5.12
49	5.06	5.24
50	5.37	4.94
51	4.79	4.86

ND = not determined

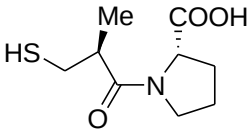
2.2.5 Dual inhibition of ACE and NEP

Chodjania *et al*. described a vasopeptidase inhibitor [(2*S*)-2-{(2*S*,3*R*)-2-thiomethyl-3-phenylbutanamido}propionic acid] S21402 (**54**) for the dual inhibition of ACE and NEP. Increased plasma levels of atrial natriuretic peptides (ANPs) ($P < 0.05$)

and urinary cyclic guanosine monophosphate (cGMP) ($P < 0.001$) showed NEP inhibition whereas increased renin concentration ($P < 0.001$) proved ACE inhibition after the administration of **54**. Compound (**54**) increased urinary sodium excretion ($P < 0.05$) without hindering blood pressure and creatinine clearance when compared with captopril (**55**).[110]



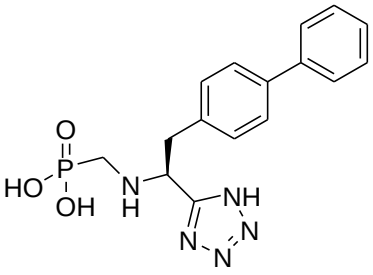
(**54**)



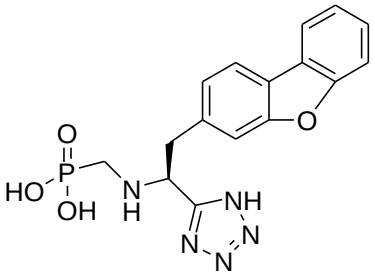
(**55**)

2.2.6 Dual inhibition of NEP and ECE

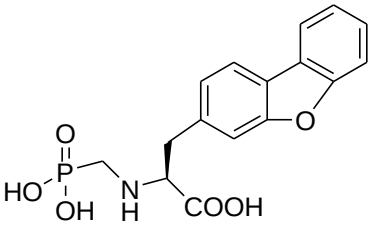
Lombaert *et al.* designed and synthesized some derivatives (**57** & **58**) by modifying the lead molecule CGS 26303 (**56**) in order to improve its dual inhibitory activities for NEP and ECE. They replaced its biphenyl substituent by 3-dibenzofuranyl moiety which improved ECE selectivity with simultaneous inhibition of NEP. These derivatives were evaluated in the *in vitro* and *in vivo* models.[111]



(**56**)



(**57**)

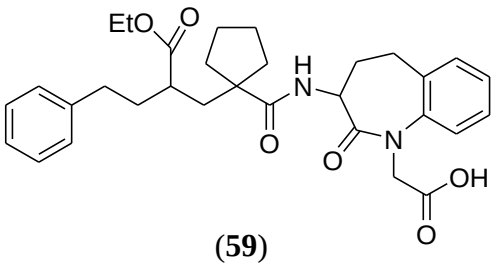


(**58**)

Comp.	IC_{50} (nM)	
	ECE	**NEP**
56	410	1
57	6	19
58	22	2300

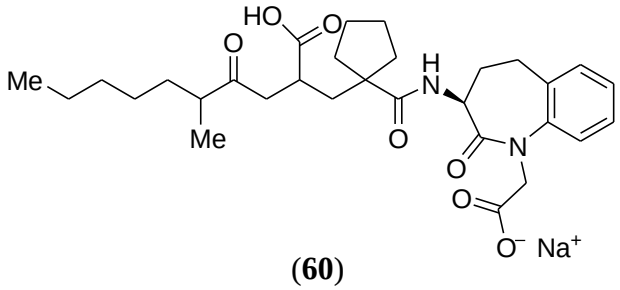
Dickstein *et al.* from Solvay Pharmaceutical Ltd., studied dual inhibitory activities of 2-[1-(carboxymethyl-2-oxo-2,3,4,5-tetrahydro-1*H*-benzo[b]azepin-3-ylcarb-amoyl)cyclopentyl-methyl]-4-phenylbutyric acid ethyl ester (**59**), for NEP and ECE. The

increased levels of natriuretic peptides and big ET-1 (an inactive precursor of ET-1) in plasma confirmed the combined inhibition of NEP and ECE which could be beneficial in heart failure by decreasing the filling pressure in heart.[112]



(**59**)

Nelissen *et al*. reported synthesis and pharmacological evaluation of 2-{[1-(3*S*)-1-(carboxymethyl)-2-oxo-2,3,4,5-tetrahydro-1*H*-1-benzazepin-3-ylaminocarbonylcyclo-pentyl]-methyl-4-[3-(methylamino)propyl(methyl)amino]}-4-oxobutanoic acid (SOL1) (**60**) as dual inhibitor for NEP and ECE. In the *in vitro* evaluation at neutral pH, **60** exhibited a potent inhibition against NEP whereas somewhat weak against ECE with IC_{50} values of 25 and 3200 nM respectively. On the other hand in the *in vivo* evaluation, **60** showed remarkable inhibition of NEP (ED_{50} 0.03 mg/kg) with simultaneous inhibition of ECE (ED_{50} 1.9 mg/kg), corresponding to plasma EC_{50} of 0.1 and 4.6 μM, respectively. Compound (**60**) was demonstrated as highly water soluble (>500 mg/ml) with improved volume of distribution (0.7 L/kg) in its pharmacokinetic studies.[113,114]

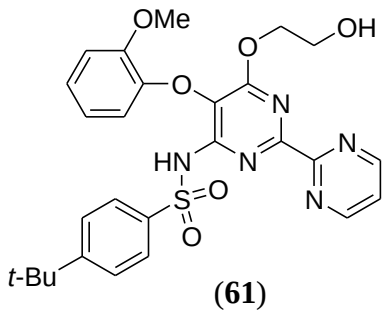


(**60**)

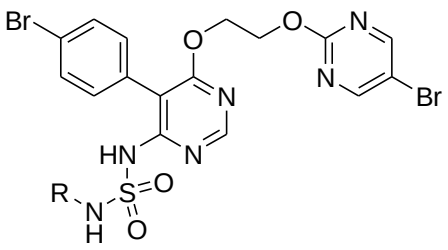
2.2.7 Combined antagonism for ET_A and ET_B receptors

Bolli *et al*. designed and synthesized a series of pyrimidine derivatives taking bosentan (**61**) as a lead molecule and reported some potent dual ET_A- and ET_B-receptor antagonists with enhanced oral bioavailability. This work led to the discovery of a potent dual (ET_A and $ET_{B)}$ antagonist (**62**) (macitentan, ACT-064992) with improved *in vivo*

efficacy and pharmacokinetics. Compound (**62**) has completed phase-III clinical trials for the pulmonary arterial hypertension. The designing strategy was based on the replacement of *tert*.butylbenzene moiety from bosentan (**61**) with alkylsulfonamide side chain. Improved binding affinities with ET_B-receptor and increased oral bioavailability rendered the novel derivatives more valuable over the parent congeners. *In vivo* de-alkylation of the novel compounds (**62** and **63**) yielded a major metabolite (**64**) formed from the pharmacologically active congeners, which displayed a sustained activity.[115]



(**61**)

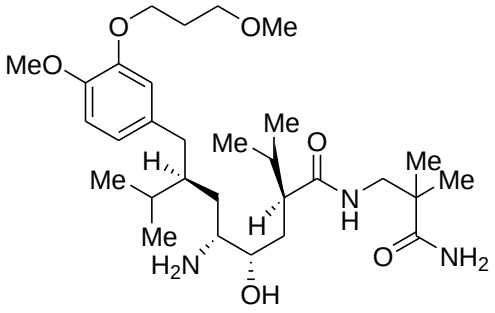


(**62**) R = *n*-Propyl
(**63**) R = *n*-Butyl
(**64**) R = H

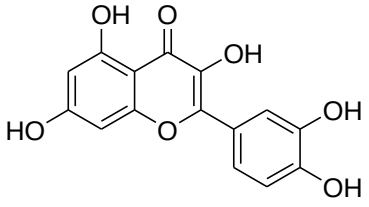
Comp.	**IC_{50} (nM)**	
	ET_A-receptor	**ET_B-receptor**
61	45	202
62	0.5	391
63	0.3	158
64	3.4	987

2.2.8 Simultaneous inhibition of ACE, renin and aldosterone

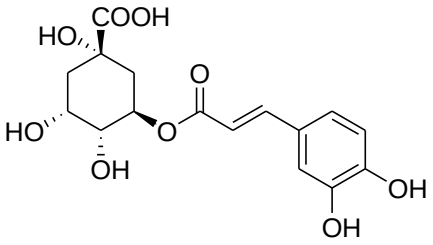
Sanderson *et al*. designed and synthesized some derivatives (e.g. **70** & **71**) of naturally occurring caffeic acid phenethyl ester (CAPE) (**69**) and biologically evaluated them for their anti-proliferative activities in prostate cancer cells. Among them, some derivatives demonstrated modulation of RAAS as reported by Bhullar *et al*.[116] through simultaneous inhibition of ACE, renin and aldosterone system. Comparisons were made with standards like aliskiren (**65**), captopril (**55**), quercetin (**66**), chlorogenic acid (**67**), caffiec acid (**68**) and CAPE (**69**).



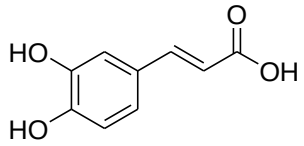
(**65**)



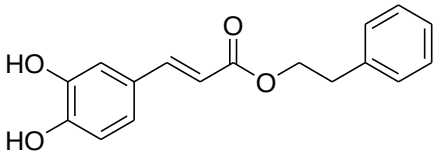
(**66**)



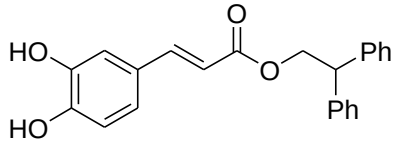
(**67**)



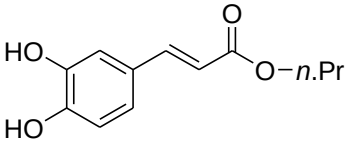
(**68**)



(**69**)



(**70**)



(**71**)

Comp.	IC_{50} (µM)		Aldosterone level (pg/mL) (SHR control = 891.81)
	Renin inhibition	ACE inhibition	
65	0.1	-	-
55	1108.04	1.02	530.59
66	971.78	9.85	569.56
67	730.83	21.53	598.35
68	5704.12	430.01	679.51
69	556.58	131.73	862.25
70	228.80	9.14	670.84
71	2846.30	1017.19	574.41

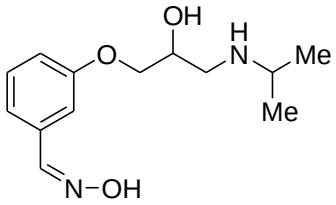
Compound (**70**) exhibited the strongest renin inhibition (IC_{50} = 229 µM) among all of the evaluated compounds ($P \leq 0.05$) and it was found to be 47 folds more potent than CAPE for ACE inhibition (IC_{50} = 9.1 µM). Compound (**71**) was a potent inhibitor of

aldosterone production. They also proved using human fibroblasts (WI-38 cells) that these derivatives were safer than clinically used drug captopril (**55**).[116]

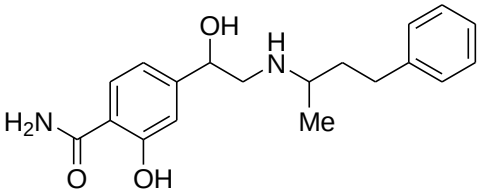
Rong *et al.* isolated an antihypertensive peptide (Gly-His-Ser or GHS) from 3 kDa fraction of rapeseed protein digest of pepsin and pancreatin which showed potent inhibition of both ACE and renin activities, simultaneously. GHS was 1.5 fold more potent for ACE inhibition (IC_{50} 0.52 ± 0.01 mg/mL) and 3.5 folds for renin inhibition (0.32 ± 0.01 mg/mL) than its parent 3 kDa permeate of pepsin digest.[117]

2.2.9 α-Adrenoceptor's (α_1) antagonism along with β_1, β_2 and β_3 modulating effects

Chaudhary *et al*. reported dual antagonistic role of PP-17 (3-(2-hydroxy-3-isopropylaminopropoxy)benzaldehydeoxime) (**72**) for α- and β-adrenoceptors through *in vitro* and *in vivo* experiments. Interestingly the projected compounds showed antagonistic activities on different subtypes of α- and β-adrenoceptors. Compound (**72**) showed balanced antagonistic activities on both α- and β-adrenoceptors subtypes with pA_2 values of 7.0, 5.7, 6.0, and 7.0 for β_1, β_2, β_3 and α_1-adrenoceptors respectively. The selectivity of compound (**72**) for β_1 and α_1-receptors was comparable with labetalol (**73**) which was having the pA_2 values of 7.9, 7.1, 6.5 and 7.5 against β_1, β_2, β_3 and α_1-receptors respectively.[118]



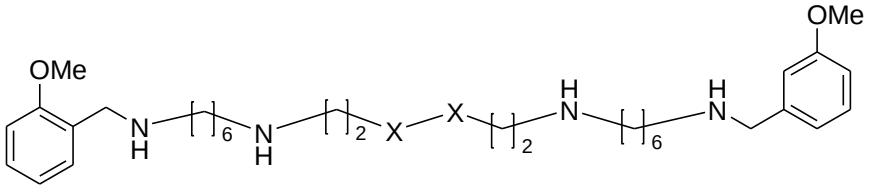
(**72**)



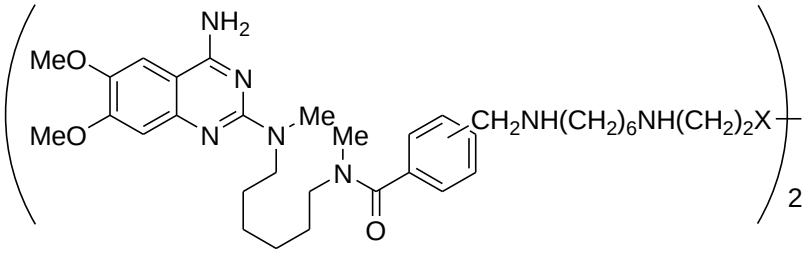
(**73**)

2.2.10 Multiple antagonism for α_1-receptor subtypes

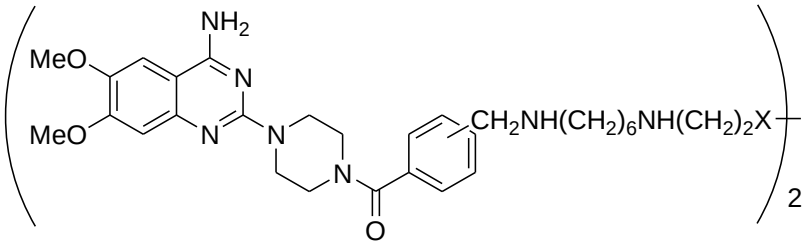
Bolognesi *et al*. designed and synthesized some hybrid tetraaminedisulfides (**76** & **77**) by merging the structural elements of prazosin (**19**) and benextramine (**74**) or its carbon analog (**75**) as irreversible α_1/α_2-receptors antagonists. Their prime goal was to determine the effect of increased electronic properties or lipophilic nature of phenyl ring on selectivity and affinity for α_1-receptor subtypes (α_{1A}, α_{1B}, α_{1D}). These hybrids were



(74) X = S
(75) X = CH_2



(**76-80**)



(**81-83**)

Comp.	Position of side chain on phenyl ring	X	pK_b (pIC_{50})		
			α_{1A}	α_{1B}	α_{1D}
19	-	-	8.99	8.74	9.71
74	-	-	4.79	4.17	5.30
75	-	-	5.32	6.01	6.21
76	3	S	7.34	5.76	8.34
77	4	S	5.33	5.67	8.50
78	3	CH_2	8.48	7.41	9.30
79	2	CH_2	8.17	7.22	8.47
80	4	CH_2	8.53	7.56	7.90
81	2	CH_2	7.74	6.80	7.94
82	3	CH_2	7.29	6.50	7.43
83	4	CH_2	6.97	7.16	7.47

biologically assessed through functional antagonism assays for α_1-receptor subtypes using isolated vas deferens (α_{1A}), spleen (α_{1B}) and aortic (α_{1D}) strips in rat model. Some derivatives (**78-83**) of carbon analog (**75**) were also included in their study to determine

the role of disulfide bridging on the binding affinities for α_1-receptor subtypes. Hybrid compounds having a disulfide bridge showed noncompetitive antagonism on α_{1A} and α_{1B} subtypes while competitive antagonism on the α_{1D} subtype. Some of the compounds (**76-78**) emerged as lead molecules for the assessment of α_1-receptor antagonism.[119]

On the basis of these outcomes they made some predictions about the binding sites of α_{1A}, α_{1B} and α_{1D} subtypes as given below:

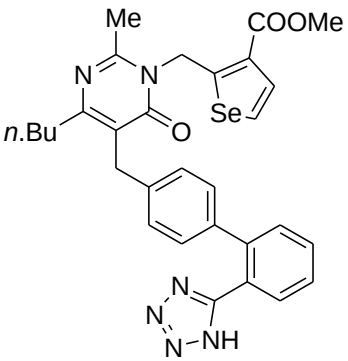
- Presence of thiol (-SH) moiety in α_{1A} and α_{1B} subtypes would influence the binding affinities towards the disulfide moiety of the antagonists; probably it was not available in the α_{1D} subtype.
- Replacement of the furan ring of compound (**19**) with a phenyl moiety significantly increased the selectivity toward α_1-receptor subtype rather than the α_2-receptor.
- The type of substituent and their position on the phenyl ring would affect the affinity and selectivity for α_1-receptor subtypes.
- Diamino moiety showed positive interactions with the receptor subtypes.

2.2.11 AT_1-antagonism along with antioxidant activity

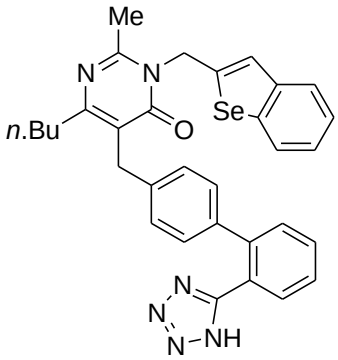
Vascular oxidative stress is a coherent problem during the development of cardiovascular diseases. Thus, a drug having antioxidant properties with blood pressure lowering effects would be in demand for the treatment of hypertension.

Tan *et al*. synthesized some analogs of milfasartan (**84**) and eprosartan (**85**) as AT_1 antagonists by attaching nitroxide moieties on the pyrimidone skeleton. The derivatives were evaluated biologically for their antihypertensive activity along with antioxidant ability through various *in vitro* and *in vivo* evaluations. Among them, compounds (**86** and **87**) showed improved AT_1 antagonistic activities with pK_B estimates in the range of 6.2-9.1 which were comparable to milfasartan (**84**) and eprosartan (**85**). In order to improve the water solubility of the compounds (**86** & **87**) their sodium salts (**88** & **89**) respectively, were also prepared and evaluated whereby **89** was found to reduce free radical generation and vascular injuries significantly.[120]

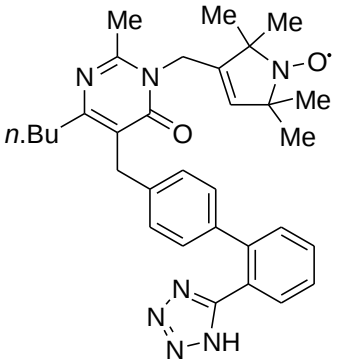
Nicotinamide adenine dinucleotide phosphate oxidase (NADPH) enzyme complex was used to generate superoxide moieties in rat aortic strips in the presence or absence of compounds (**88** and **89**) which were detected using lucigenin-enhanced chemi-luminescence.[121,122] NADPH-derived superoxide counts were found 2009 mg^{-1} of dry tissue weight for the control tissues. Compound (**88**) reduced the superoxide counts to



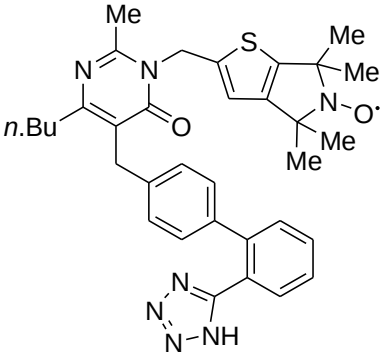
(**84**)



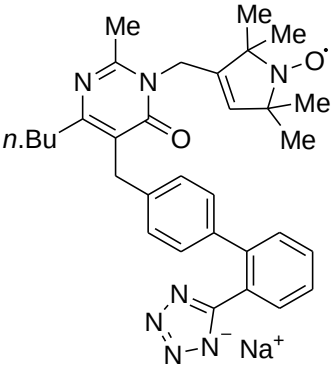
(**85**)



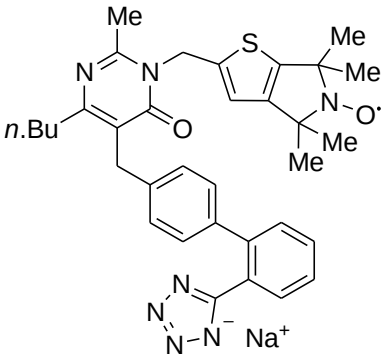
(**86**)



(**87**)



(**88**)



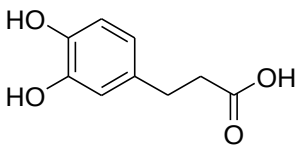
(**89**)

Comp.	pK_B estimates	Reduction in superoxide counts at different concentrations (mg/ dried tissue weight)		
		1 µM	3 µM	10 µM
84	9.5	-	-	-
85	8.4	-	-	-
86	9.1	-	-	-
87	8.4	-	-	-
88	9.1	991	310	115
89	8.4	-	535	221

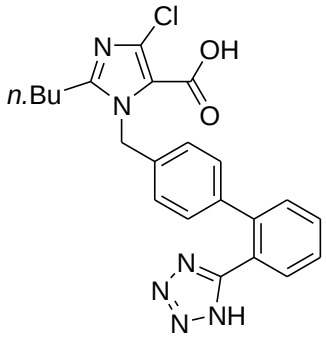
991, 310 and 115 mg^{-1} at the concentrations of 1, 3 and 10 µM respectively whereas compound (**89)** reduced the counts to 535 and 221 mg^{-1} at concentrations of 3 and 10 µM

respectively. These results showed that both of these compounds possessed antioxidant properties but, compound (**89**) was somewhat more potent than **88** as an antioxidant.[123]

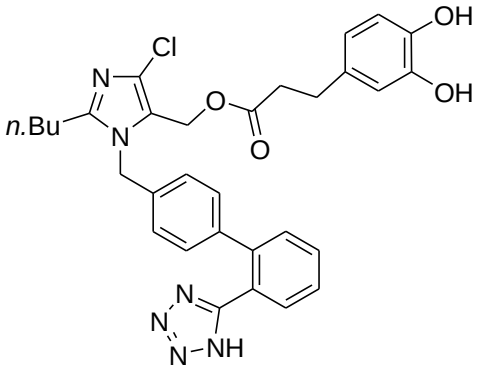
Garcia *et al*. reported a series of losartan-hydrocaffeic acid hybrids. They synthesized these compounds by attaching of hydrocaffeic acid (**90**) to the 5th position of the alkyl chain of losartan (**13**) using ester, amide and amine as linker moieties in order to



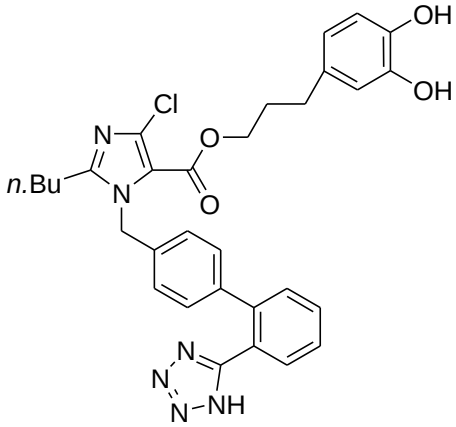
(**90**)



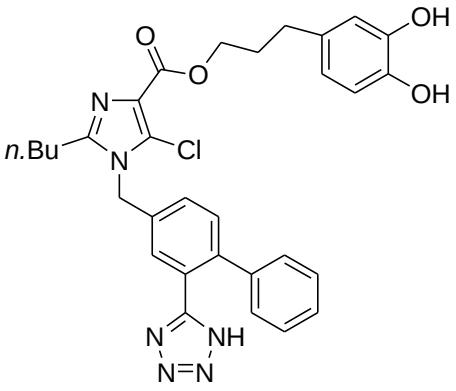
(**91**)



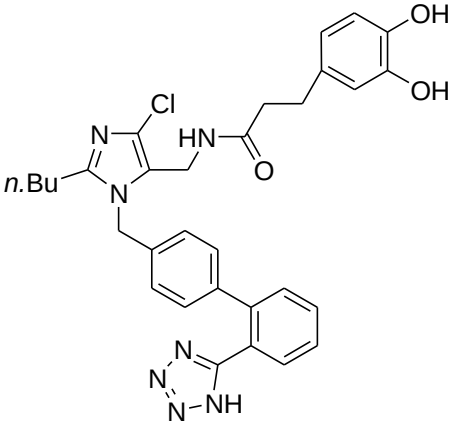
(**92**)



(**93**)



(**94**)



(**95**)

Comp.	TEAC (mM) (n = 8)	% Inhibition (n = 5)	Systolic arterial pressure (SAP) (mm Hg)			
			P_0	P_1	P_2	P_2-P_1
13	0.03	93	126	171	157	13
90	0.36	85	-	-	-	-
91	0.03	-	-	-	-	-
92	0.31	94	139	175	145	29
93	0.10	88	127	175	167	9
94	0.24	95	136	177	163	14
95	0.27	90	131	175	162	13

P_0 = Basal SAP; P_1 = SAP after one week treatment with Nω-nitro-$_L$-arginine methyl ester hydrochloride ($_L$-NAME); P_2 = SAP after three days treatment with hybrid drugs.

obtain improved antioxidant efficacy along with AT_1 antagonistic activity. Most of them emerged as 3 to 9 times more potent antihypertensive agents in comparison to losartan (**13**) or its metabolite (**91**) due to the additional antioxidant activity. Compound (**92**) was the most potent antihypertensive agent with improved antioxidant properties. Compounds (**93-95**) were also reported as effective modulators for raised blood pressure due to their dual mechanism. The antioxidant ability was performed using the ABTS method and expressed as TEAC (Trolox Equivalent Antioxidant Capacity) which referred the antioxidant equivalent concentration in mM.[123]

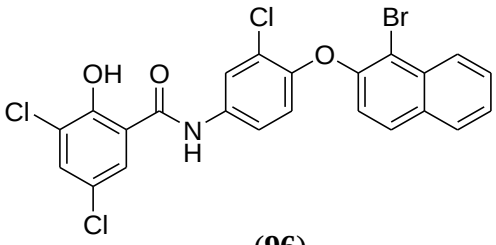
On the basis of comparison with the structure of losartan (**13**), structure activity relationships were developed between the measured antioxidant activity and the type of linker for aromatic ring or the type of substituent at different positions, such as:

- Presence of hydroxy groups, on the phenyl moiety of hydrocafeic acid, is mandatory for remarkable dual (AT_1-antagonistic and antioxidant) activities.
- Between phenol and the heterocyclic functional group, at least one methylene linker is mandatory.
- Linkers with ester and amide functionalities, offered potent dual acting hybrids.

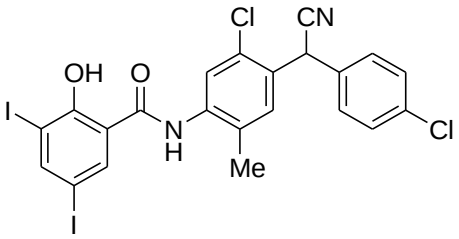
2.2.12 Dual effects through NaCl reabsorption and vasodilation

Kikuchi *et al*. observed the hypertensive effect of STE20/SPS1-related proline-alanine-rich protein kinase (SPAK). SPAK activated SLC12A type of transporters such as Na^+-Cl^- co-transporters (NCC) and Na^+-K^+-$2Cl^-$ co-transporters (NKCC1). These transporters imparted their role in the regulation of blood pressure through dual mechanism as re-absorption of salt (NaCl) and vasoconstriction. They screened more

than 20000 small molecules to develop potent SPAK inhibitors. As a result, they discovered stock-1S-14279 (**96**) and closantel (**97**) using drug repositioning strategy as potent SPAK inhibitors.[124]



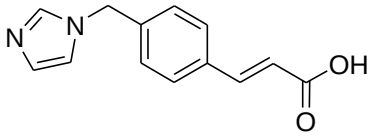
(**96**)



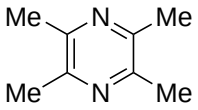
(**97**)

2.2.13 Platelets anti-aggregation along with antioxidant activity

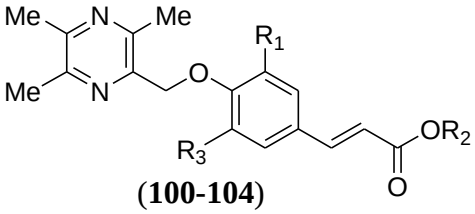
Chen *et al.*[125] designed and synthesized ligustrazinyloxycinnamic acid derivatives (**100-108**) which were biologically evaluated by *in vitro* assay for platelet aggregation inhibitory effects along with their protective effect against hydrogen peroxide (H_2O_2) ind-



(**98**)

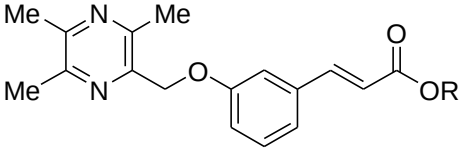


(**99**)



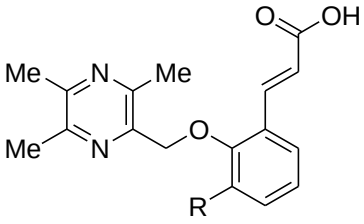
(**100-104**)

(**100**) R_1 = H; R_2 = H; R_3 = H, (**103**) R_1 = OMe; R_2 = Et; R_3 = H,
(**101**) R_1 = OH; R_2 = H; R_3 = H, (**104**) R_1 = OMe; R_2 = Et; R_3 = OMe
(**102**) R_1 = OMe; R_2 = H; R_3 = H,



(**105, 106**)

(**105**) R = H, (**106**) R = Et



(**107, 108**)

(**107**) R = H, (**108**) R = OMe

Comp.	Platelet aggregation inhibition IC_{50} values (mM)	Antioxidant activity EC_{50} values (mM)
98	0.360	-
99	-	0.600
100	1.402	0.086
101	0.176	0.046
102	0.157	0.295
103	0.449	0.109
104	0.631	2.308
105	0.161	0.020
106	0.628	4.333
107	0.054	0.499
108	0.522	3.592

uced oxidative damage on ECV-304 cells. Compound (**105**) among them, exhibited remarkable activities on both the targets (antioxidant; EC_{50} = 0.020 mM, platelets anti-aggregation; EC_{50} = 0.161 mM) simultaneously. Biological evaluation for platelet anti-aggregation activity using ozagrel (**98**) (IC_{50} = 0.360 mM) as standard indicated the compounds (**102**, **105** and **107**) to be the most potent ones with IC_{50} values of 0.157, 0.161 and 0.054 mM, respectively. Compounds (**103**, **104**, **106** and **108**) were also effective (IC_{50} <1.0 mM). SAR studies proved that replacement of the carboxyl group with the ester group reduced the anti-aggregation potencies by many folds.

On the other hand, some compounds (**100**, **101** and **105**) exhibited notable antioxidant activity with EC_{50} values of 0.086, 0.046 and 0.020 mM, respectively in comparison to tetramethylpyrazine (ligustrazine) (**99**) (EC_{50} = 0.60 mM) on the damaged ECV-304 cells.[125]

2.2.14 Dual α_1 and 5-HT_{1A}- antagonisms

Betti *et al*. synthesized a series of alkoxyarylpiperazinylalkylpyridazinone derivatives and evaluated them on α_1-, α_2- and 5-HT_{1A}-receptors, simultaneously. Among them, compound (**111**) was found about 5-times (0.052 nM) more potent than prazosin, towards α_1-receptor but it failed to bind to α_2-receptor. Interestingly, compound (**113**) emerged as a dual antagonist for the 5-HT_{1A} and α_1-recptors with an affinity ratio of 119 in favour of α_1-recptors.[126]

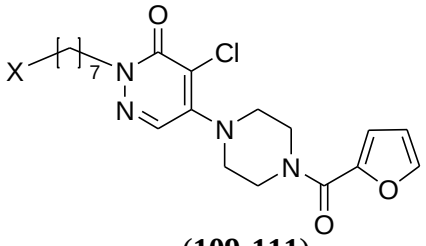
Correlating the structural features of the synthesized compounds and their biological data for the α_1-receptor binding affinity, the following suggestions were made:

i) The size of the *o*-alkoxy group is important for the binding affinity *i.e.* with an increase in the size binding affinity also increases. Such as compounds (**111** and **119**) among the synthesized compounds showed higher binding affinities (0.05 and 0.08 nM, respectively).

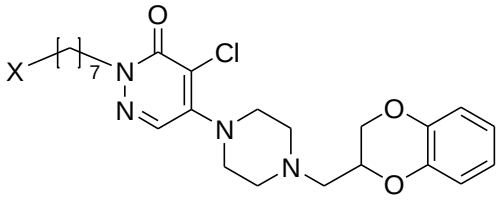
ii) Binding affinity is not affected by modifying the substituents present on the pyridazinone ring as showed by compounds (**112** and **113**).

iii) When comparisons were made among the furoyl, benzodioxane and phenyl derivatives, some interesting results came out, such as all those compounds having *o*-methoxy and *o*-ethoxy substituents were found with comparable affinities (**109**, **110** Vs **112**, **113**, **115**, **116** and **117**, **118** Vs **120**, **121**) whereas compounds bearing the isopropoxy group in the furoyl category were found four (**119** Vs **122**) to six (**111** Vs **114**) times more potent than their benzodioxane and phenyl counterparts.

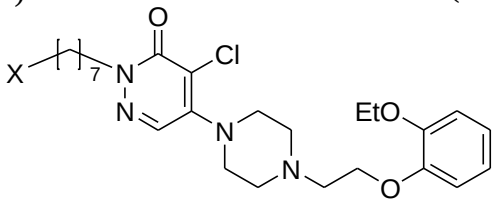
The binding affinity for α_2-receptor got increased with increase in bulk of the alkoxy group in the furoyl congeners while it got decreased with bulkiness of the alkoxy groups in the benzodioxane and alkoxyphenoxyethyl congeners.



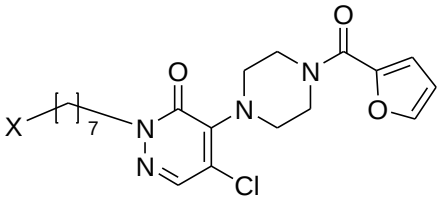
(**109-111**)



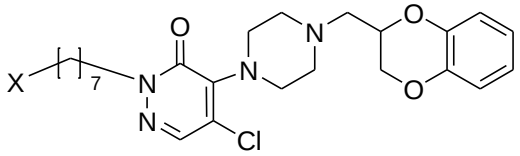
(**112-114**)



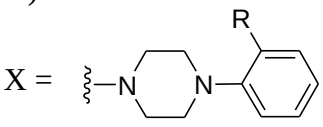
(**115**, **116**)



(**117-119**)



(**120-122**)



Comp.	R	K_i (nM)		
		α_1-	**α_2-**	**5-HT_{1A}**
109	OMe	1.9	520	ND
110	OEt	0.5	4.0	ND
111	O*i*.Pr	**0.052**	0.56	**0.90**
112	OMe	1.68	0.85	11.10
113	OEt	**0.42**	2.16	**49.80**
114	O*i*.Pr	0.31	3.34	2.47
115	OMe	0.55	1.59	0.82
116	OEt	0.43	2.04	1.32
117	OMe	0.37	1.23	0.42
118	OEt	0.23	0.80	0.35
119	O*i*.Pr	**0.08**	0.66	**1.16**
120	OMe	0.54	1.45	0.40
121	OEt	0.43	2.40	1.02
122	O*i*.Pr	0.32	13.40	1.52

ND = not determined

2.2.15 AT_1 and AT_2- receptor antagonists

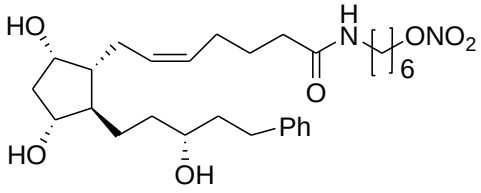
Walsh *et al*. synthesized and biologically evaluated some α-phenoxyphenylacetic acid derivatives as potent AT_1- and AT_2-receptor antagonists. SAR studies revealed that substituting *n*-propyl moiety on the central aromatic ring of α-phenoxyphenylacetic acid, gave compound (**14**) which significantly displayed low nanomolar potencies (AT_1; IC_{50} = 11 and AT_2; IC_{50} = 47 nM) at both the receptors.[127]

Chang *et al*. reported some orally active non-peptides which blocked the effects induced by the interactions between AII and its (AT_1 and AT_2) receptors. They worked on trisubstituted-1,2,4-triazolinone biphenylsulfonamides with the aim to reduce AT_2/AT_1 potency ratio (IC_{50} ratio) from the original ≥ 10, *i.e.* increasing AT_2 antagonistic

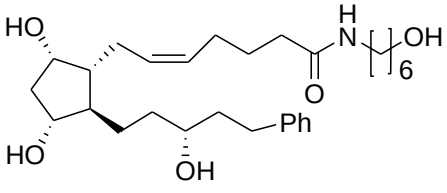
activity with equal enhancement in AT_1 affinity. Compounds, having ethyl moiety at C-5 position of triazolinone and a 3-fluoro substituent at N^4-biarylmethyl scaffold have been found with AT_2/AT_1 potency ratio of ≤1. Among the reported series, compound (**15**) showed the potency in subnanomolar range on both the (AT_1- and AT_2-) receptors and reduced adrenal AT_2/AT_1 potency ratio upto 1. Compound (**15**) offered an excellent intravenous activity at a dose of 1 mg/kg and also showed improved oral bioavailability at a dose of 3 mg/kg with long duration of action in a conscious rat model. They revealed that keto (-C=O) functionality on the N^2-position of the aromatic ring was favorable instead of the NH of the amide at this position in order to achieve an efficient AT_2 binding.[128]

2.2.16 Dual effects through NO mediated vasodilation and $PGF_{2\alpha}$ inhibition

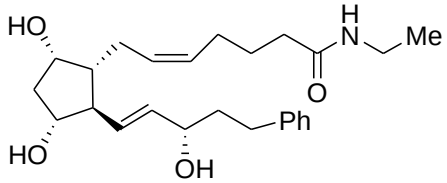
Impagnatiello *et al*. reported NCX-139 (**123**) a hybrid molecule consisting of latanoprostamide and an NO donating moiety. The *in vitro* studies showed that compound (**123**) was more effective than its des-nitro analog (**124**) for lowering the ocular hypertension. Compound (**123**) produced NO-mediated vasodilatation of pre-contracted rabbit aortic rings with EC_{50} value of 0.70 µM while its parent des-nitro analog (**124**) was ineffective. On the other hand it also showed binding affinities (0.77 µM) for $PGF_{2\alpha}$ like bimatoprost (**125**) (IC_{50} = 3.07 µM) and latanoprost acid (**126**) (IC_{50} = 0.48 µM).[11]



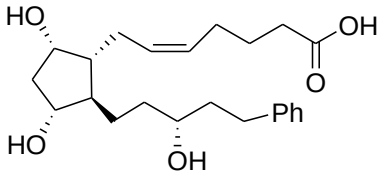
(**123**)



(**124**)



(**125**)



(**126**)