Introduction

1.1 Hypertension

As the thesis pertains to research work carried out for the development of multitargeted ligands as potential antihypertensive agents, it is in order to introduce the reader about hypertension and related aspects. Hypertension is recognized as one of the leading risk factors for human morbidity and mortality. On a worldwide basis hypertension has been ranked on the top as a cause of disability adjusted life years.[1] Recently, the global prevalence of hypertension (systole/diastole ≥140/90 mmHg) was estimated for the year 2000 and the data was used to predict the global prevalence of hypertension by 2025 (Fig. 1).[2] More than 25% of the world's adult population was hypertensive by the afore-mentioned criteria in 2000. The estimated total number of people with hypertension in 2000 was 972 million, and this is projected to increase by 60% to a total of 1.56 billion by 2025, i.e., 29% of the worldwide adult population.[3]

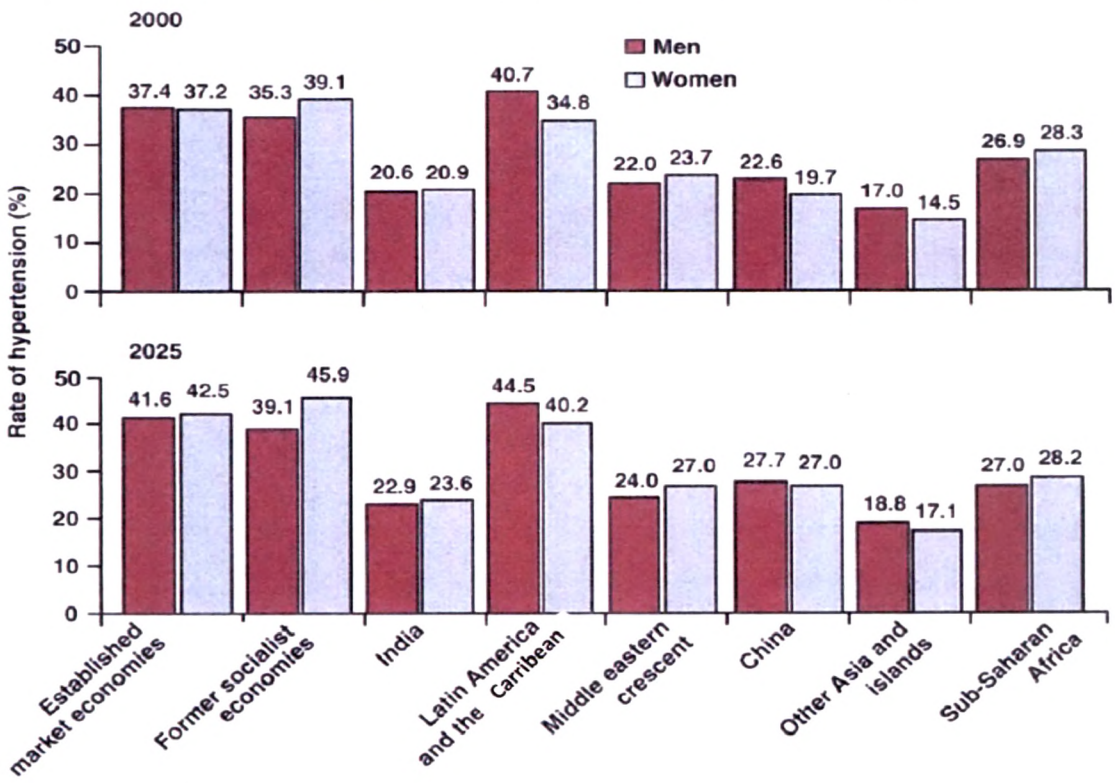


Figure 1: Frequency of hypertension in people of ages 20 years and older in the world regions and genders in 2000 (upper panel) and projected to be in 2025 (lower panel).

Hypertension is a major risk factor for myocardial infarction, congestive heart failure, stroke and end-stage renal disease.[4] Blood pressure is derived from the hemodynamic properties of closed systemic circulation. Therefore the tension on the walls of blood vessels depends on several factors, like:

(a) The pumping function of the heart

(b) The total blood volume

(c) The size, structure and distensibility of the vascular tree and

(d) Other factors like reflex and neurohumoral feedback systems which in turn may interfere with a, b and c.

Thus, hypertension is influenced by both, function and structure of blood vessels. As a consequence of elevated blood pressure arterial elasticity is reduced and wall damage appears that can lead to cholesterol and fat deposition on these lesions and eventually to obstruction of the vessels. This is the basis of most of the target organ damages induced by hypertension. Another consequence can be an increase in vascular resistance which forces the pumping activity of the heart to maintain nutrients and oxygen distribution. This work overload for the heart may induce the development of cardiac hypertrophy, an increase in cardiac mass and thickness.[5]

Some patients may "inherit" abnormalities that make them prone to the development of hypertension as well as a complex series of cardiovascular disease risk factors. These include elevated lipids, increased left ventricular hypertrophy (LVH), arterial stiffening, insulin resistance, renal function abnormalities and neuroendocrine changes. Studies assessing both arterial structure and function have shown reduced arterial compliance in normotensive subjects with a family history of hypertension.[6,7] Insulin resistance has been shown to occur in approximately 50% of hypertensive patients.[8] Elevated blood pressure has been implicated as a cause for renal dysfunction in hypertensive patients. The sympathetic nervous system (SNS) and the renin-angiotensin system (RAS) are believed to be pivotal in the pathogenesis of hypertension. Interruptions of these systems effectively reduce blood pressure.[9]

The difficulty in controlling hypertension is related, at least in part, to the complex pathogenesis of hypertension and related cardiovascular diseases. Multiple signaling pathways and redundant feedback mechanisms, both positive and negative, contribute to the hypertensive disease process, which is further confounded by the interrelationship of hypertension with associated diseases such as diabetes and renal dysfunction.

1.2 Drug targets for the management of hypertension

Hypertension is, by definition, a hemodynamic disorder. The major hemodynamic finding associated with higher levels of blood pressure is a rise in peripheral vascular resistance. This observation led to the discovery and development of increasingly complex and targeted vasodilators, although many of the earlier antihypertensive drugs, by virtue of their actions blocking the sympathetic nervous system, had a vasodilator component to their mode of action. The first non-specific vasodilator was hydralazine.[10] Approaches made in the search of effective antihypertensive agents revealed more systems and newer targets as discussed below[11-13]:

- The sympathetic nervous system (SNS) (since 1954) was then explored for the treatment of hypertension. SNS is involved in the homeostatic regulation of a wide variety of functions such as heart rate, force of contraction of the heart, vasomotor tone and ultimately blood pressure. The sympathetic nervous system is subdivided into the α and β subsystems. β_1 Receptor blockade results into decreased cardiac output while α receptor blockade caused peripheral vasodilatation.
- Diuretics provide a means of forced diuresis to increase the excretion of water from body. Kidney is a vital organ in the maintenance of fluid volume. There are many classes of diuretics like thiazides, loop and potassium sparing etc.
- Calcium Channel Blockers (CCBs) (1980) are very effective antihypertensive agents that reduce blood pressure primarily through arteriolar vasodilatation.
- Renin Angiotensin System (RAS) is an important target for renal and cardiovascular protection. Angiotensin converting enzyme inhibitors (ACEIs) were successfully developed in mid 1980. Later on, angiotensin receptor blockers

(ARBs) were developed (1990). Now a days, renin inhibitors are also available (2010).

The search for the effective control of blood pressure revealed more targets like:

- Aldosterone is a potent mineralocorticoid which promotes Na^+ reabsorption causing increase in water level. Aldosterone receptor antagonists (ARA) act at the mineralocorticoid receptor level by competitively inhibiting aldosterone binding while Aldosterone Synthase Inhibitor (ASIs) inhibit the action of aldosterone synthase.[14]
- Endothelin 1 (ET_1) is a twenty one amino acid vasoactive peptide that is released predominantly from vascular endothelium[15] and is synthesized by a variety of cell types including vascular smooth muscles, cardiomyocytes and cardiac fibroblasts.[16] Endothelin stimulates potent vasoconstriction and cell proliferation through activation of endothelin A receptor. Endothelin receptor antagonists are useful in treatment of pulmonary hypertension.[17]
- Prostacyclin, a metabolite of arachidonic acid, has vasoprotective effects including vasodilation, platelet antiaggregation, and inhibition of smooth cell proliferation.[18, 19] Prostacyclin analogues are antagonists useful for the treatment of pulmonary hypertension.[20]
- The nitric oxide (NO)/soluble guanylate cyclase (sGC)/cyclic guanosine-3',5'-monophosphate (cGMP) pathway plays an important role in cardiovascular regulation by producing vasodilation and inhibiting platelet aggregation, and vascular smooth muscle proliferation. Soluble guanylate cyclase activators increase intracellular cGMP concentrations resulting in relaxation of the smooth muscle of the vasculature.[21]
- Phosphodiestarase (PDE) inhibitors can prolong or enhance the effects of physiological processes mediated by cAMP or cGMP by inhibition of their degradation by PDE. These phosphodiesterase inhibitors are used primarily as remedies for erectile dysfunction and have medical applications such as treatment of pulmonary hypertension.[20]

1.3 Monodrug therapy

A great deal of clinical research over the past few decades has attempted to answer the seemingly critical question, "What is the best drug for hypertension?" Long-term clinical trials have successfully demonstrated the efficacy of different classes of drugs including angiotensin converting enzyme (ACE) inhibitors, calcium channel blockers (CCBs), angiotensin receptor blockers (ARBs), β_1-blockers (BBs), α_1-blockers, aldosterone antagonists and diuretics. The report of JNC VII provided a list of oral antihypertensive agents (**Table 1**).[23]

Table 1. Oral antihypertensive agents

Sr. No.	Class	Drugs
1	ARBs	Losartan, Valsartan, Olmesartan, Telmisartan, Candesartan, Irbesartan, Eprosartan
2	ACEIs	Captopril, Ramipril, Benzapril, Enalapril, Fosinopril, Lisinopril, Trandolapril, Perindopril, Quinapril, Moexipril,
3	CCBs	Amlodipine, Felodipine, Nicardipine, Nifedipine, Nisoldipine Diltiazem, Verapamil
4	β_1- Blockers	Atenolol, Betaxolol, Bisprolol, Metoprolol, Nadolol, Propranolol, Timolol
5	α_1- Blockers	Prazosin, Doxazosin, Terazosin
6	Aldosterone antagonists	Eplerenone, Spironolactone
7	Diuretics	Hydrochlorothiazide, Chlorothiazide, Chlorthalidone, Polythiazide, Indapamide, Metolazone, Bumetamide, Furosemide, Torsemide, Amiloride, Triamterene
8	Direct vasodilators	Hydralazine, Minoxidil
9	Combined α and β blockers	Carvedilol, Labetalol
10	Central α_2 agonists	Clonidine, Methyldopa, Reserpine, Guanfacine

Hypertension is a risk factor and may associate with several disorders or conditions. The current antihypertensive therapy is able to treat the hypertension in patients with different disorders or conditions. Many drugs are reported to be effective in

treating hypertensive patients with different disorders or conditions. The systems, their targets and benefits in treating hypertension associated with other disorders or conditions are discussed below.

1.3.1 Sympathetic Nervous System (SNS)

The sympathetic nervous system is involved in the homeostatic regulation of a wide variety of functions such as heart rate, force of contraction of the heart, vasomotor tone and ultimately blood pressure. The sympathetic nervous system is subdivided into the α and β subsystems. The hyperactivity of this system leads to various cardiovascular disturbances such as hypertension, shock, cardiac failure and arrythmias, asthma, allergy and anaphylaxis. α_1 Receptor causes peripheral vasoconstriction. Commonly used antagonists are prazosin, doxazosin and terazosin. In V-HeFT 1 study, men with chronic congestive heart failure and cardiac dilatation (CT ratio > 0.55) or LVEF <45% in association with reduced exercise tolerance were treated with prazosin or placebo. Prazosin reduced all cause deaths by 6%. [24]

β_1 Receptor blockade results into decreased cardiac output. β Blockers have an important dual role to play in the management of patients with diabetic kidney disease - to help achieve target BP and to provide optimal cardioprotection in those patients who are at high risk for cardiac events. β Blockers clearly have a role in reducing CV risk in the treatment of patients with diabetic or nondiabetic kidney disease.[25]

1.3.2 Diuretics

Diuretics are effective antihypertensive drugs. Treatment with a diuretic such as hydrochlorothiazide results in a dose-dependent blood pressure reduction that levels off with higher dosages.[26] In long-term trials diuretics have been shown to reduce the incidence of stroke, congestive heart failure, coronary artery disease and total mortality from cardiovascular diseases.[27]

1.3.3 Calcium Channel Blockers (CCBs)

Calcium channel blockers are very effective antihypertensive agents that reduce blood pressure primarily through arteriolar vasodilatation. CCBs have also been shown to improve the CV risk profile to a greater degree than that expected by their BP-lowering effects alone and to provide additional advantages in terms of renal and vascular protection, reduction in new-onset diabetes cases and lack of effect on metabolic parameters.[28]

1.3.4 Renin-Angiotensin System (RAS)

RAS is an important target for renal and cardiovascular protection. A hormonal cascade regulates blood volume and arterial pressure to maintain adequate organ perfusion. Chronic RAS activation results in vascular and cardiac hypertrophy, vasoconstriction, and salt and water retention. The RAS cascade starts with the release of renin into the circulation from the juxtaglomerular cells of the kidney. Active renin in the plasma cleaves angiotensinogen (produced by the liver) to angiotensin I (ang I), which is then converted by circulating and locally expressed angiotensin converting enzyme (ACE) to angiotensin II (ang II). Most of the effects of ang II are exerted by its binding to angiotensin II type 1 receptor (AT_1). Therapeutic agents that block RAS via different mechanisms include ACEIs, ARBs and direct renin inhibitors.[29]

ARBs are an effective class of antihypertensive agents. They showed effects beyond blood pressure control. Mega-trials of ARBs in patients with hypertension have confirmed that blood pressure (BP) control with these agents reduced cardiovascular disease (CVD) morbidity and mortality in a range of patients, including those with diabetes mellitus, heart failure or left ventricular hypertrophy (LVH) and those at risk of developing heart failure following myocardial infarction.[30] The ARBs have demonstrated renoprotective efficacy in several large trials in patients with nephropathy associated with type 2 diabetes.[31-40]

ACEIs have been demonstrated to be similar to conventional standard therapy (β-blockers, diuretics or calcium channel blockers) in patients with hypertension or high risk patients with evidence of vascular disease or diabetes plus other cardiovascular risk factors.[41-45] In a systematic review of five long-term trials involving 12,763 patients with left ventricular (LV) dysfunction or heart failure (HF), treatment with ACEIs significantly reduced mortality and rates of readmission for HF and/or reinfarction versus placebo.[46]

Renin inhibitors represent a new class of drugs that suppress renin-angiotensin system (RAS) by blocking the action of renin on angiotensinogen to produce angiotensin I (**Table 2**). Aliskiren is the first direct renin inhibitor available for the treatment of hypertension. Available evidence shows that aliskiren is a potent and safe antihypertensive agent when used alone and in combination with other antihypertensive agents. To date, aliskiren has been shown to be effective in patients with stage 1 and 2 hypertension, diabetes, left ventricular hypertrophy, proteinuria or heart failure.[47-49]

Table 2. Renin inhibitors and their clinical status[50]

Sr. No.	Compound	Company	Phase of Development
1	Aliskiren	Novartis and Speedel Pharmaceuticals	Approved 2007
2	SPP635	Speedel Pharmaceuticals	Phase I
3	SPP676	Speedel Pharmaceuticals	Phase I
4	SPP1148	Speedel Pharmaceuticals	Phase I
5	VTP2799	Vitae Pharmaceuticals	Phase I
6	SPP1234	Speedel Pharmaceuticals	Preclinical

SPP635, another molecule from this category of drugs showed the safety and efficacy in male and female patients with mild-to-moderate hypertension monitored by measuring office and ambulatory blood pressure.[51]

1.3.5 Aldosterone receptor antagonists

Aldosterone, independent of ang II, has been implicated in the pathogenesis of progressive cardiovascular[52, 53] and renal disease.[54] Aldosterone antagonists have proved to be as effective as other antihypertensive drugs in the treatment of high BP [55-62] and useful in reducing a variety of cardiovascular[52] and renal[60] endpoints.

Eplerenone was found at least non-inferior to amlodipine,[63] enalapril[64] and losartan[65] in reducing blood pressure. In addition, eplerenone lowers blood pressure in patients with hypertension[66] and reduces all cause mortality in patients with heart failure when added to conventional therapy.[67] In EMPHASIS-HF study, the efficacy of eplerenone was again proved in patients with New York Heart Association class II heart failure with an ejection fraction of no more than 35%.[68]

1.3.6 Vasopressin receptor antagonists

One of the hormones that is increased in chronic heart failure is vasopressin. Vasopressin reduces free water excretion and at high concentrations, causes vasoconstriction in the peripheral vasculature. Subsequently, vasopressin acts on the renal collecting duct to cause retention of free water and a subsequent increase in blood pressure.[69-71] Out of the two receptors, V_1 subtype receptor appears primarily responsible for vasopressor activity while the V_2 subtype receptor appears to regulate the antidiuretic effects of vasopressin. The V_1 subtype receptor can be further distinguished into V_{1A} and V_{1B} (also called V_3) receptors.[72-76] Few compounds have been reported to possess V_{1A} selective (Relcovaptan, OPC-21268), V_{1B} selective (SR 121463A, SR121463B, OPC-31260, Tolvaptan, Lixivaptan, VPA-343) or both V_{1A} and V_{1B} selective (Clonivaptan, YM-471) antagonism.[77]

Two identical prospective randomized double-blind placebo-controlled trials were conducted during the inpatient period of the Efficacy of Vasopressin Antagonism in Heart Failure Outcome Study with Tolvaptan (EVEREST). Patients hospitalized with heart failure and congestion were studied. In patients hospitalized with heart failure, oral

tolvaptan in addition to standard therapy including diuretics improved many, though not all, heart failure signs and symptoms, without serious adverse events.[78]

1.3.7 Endothelin receptor antagonists

Endothelin 1 is a 21 amino acid vasoactive peptide that is released predominantly from vascular endothelium[79] and is synthesized by a variety of cell types including vascular smooth muscles, cardiomyocytes, and cardiac fibroblasts.[80] Endothelin causes potent vasoconstriction and cell proliferation through activation of endothelin A receptors on vascular smooth muscle cells, whereas endothelin B receptors are primarily involved in the mediation of vasodilatation through effects on the clearance of endothelin, inhibition of endothelial apoptosis, release of nitric oxide and prostacyclin, and inhibition of endothelin converting enzyme 1 expression.[81] The inhibitors of endothelin receptors (A or A/B), such as bosentan, darusentan, sitaxsentan, and tezosentan, represent a newer class of antihypertensive drugs in treating pulmonary arterial hypertension.[82]

In a trial, the blood-pressure-lowering effects of darusentan revealed additional benefit of reduction in blood pressure in patients who had not attained their treatment goals with three or more antihypertensive drugs.[83]

1.3.8 Prostacyclin analogues

Prostacyclin, a metabolite of arachidonic acid, has vasoprotective effects including vasodilation, platelet antiaggregation and inhibition of smooth cell proliferation.[84, 85] Prostacyclin analogues epoprostenol, reprostinil and iloprost are useful for the treatment of pulmonary hypertension.[86] The trial on epoprostenol (FIRST) for patients with class IIIb/IV congestive heart failure and decreased LVEF did not reveal effectiveness of the drug.[87]

1.3.9 Soluble guanylate cyclase activators

The nitric oxide/soluble guanylate cyclase/cyclic guanosine-3',5'-monophosphate

pathway plays an important role in cardiovascular regulation by producing vasodilation and inhibiting platelet aggregation and vascular smooth muscle proliferation.

Soluble guanylate cyclase activators increase intracellular cGMP concentrations resulting in relaxation of the smooth muscle of the vasculature. Soluble guanylate cyclase is pharmacologically activated on binding nitric oxide at a heme site bound to the protein, and then catalyses the conversion of guanosine triphosphate (GTP) to cGMP.[21]

Cinaciguat (BAY-58-2667, Bayer AG) is currently in developmental stage. In clinical trials in patients with acute decompensated heart failure, cinaciguat potently unloaded the heart, increased cardiac output and renal blood flow, and preserved renal function and sodium and water excretion without further neurohumoral activation.[88] Riociguat (BAY 63-2521) another molecule in phase III trials, possessed rapid, potent and prolonged efficacy and good tolerability in different types of pulmonary hypertension.[89]

1.3.10 Phosphodiestarase (PDE)

Phosphodiestarase can prolong or enhance the effects of physiological processes mediated by cAMP or cGMP by inhibition of their degradation by PDE. Sildenafil, vardenafil and the newer udenafil and avanafil selectively inhibit PDE_5, which is cGMP-specific and responsible for the degradation of cGMP in the corpus cavernosum.[86]

Nitric oxide is a potent vasodilator that also inhibits platelet adhesion and smooth muscle cell proliferation. Its inhalation has been shown to improve hemodynamics with pulmonary selectivity and improves exercise capacity in patients with pulmonary hypertension.[90]

1.4. Combination therapy

In spite of the availability of variety of antihypertensive agents, BP control in the general population is at best inadequate. Because of its multifactorial nature, simply

interfering with one of its pathophysiologic mechanisms by monotherapy is usually insufficient to control it. Treatment with a single antihypertensive agent will generally control BP in less than half of the patients and more than 60% of the patients require combination therapy with two or more drugs of different classes to achieve target BP, as has been observed in a number of large clinical trials [for example, ALLHAT (63%); PROGRESS (58%), INVEST (70%), INCLUSIVE (70%), LEAAD (60%) and SHIELD (74%)].[91-97] Blood pressure control is very important, as a large meta-analysis of one million hypertensive patients showed that a 2 mmHg reduction in systolic BP is associated with 7% and 10% reductions in the risk for cardiovascular and stroke deaths, respectively.[97]

Increasingly, it is being recognized that a balanced modulation of several targets can provide a superior therapeutic effect profile compared to the action of a selective ligand. The goal of antihypertensive treatment is to maximize therapeutic efficacy without significant adverse effects. Therefore, antihypertensive therapy has been directed toward improving BP control in treating patients with the available drugs by using the right combinations at optimum doses. New clinical trials are needed to determine optimal drug combinations that will also confer target-organ protection in addition to and independent of their BP lowering effects. Some poor or troublesome combinations that have been reported are β-blockers with ACEIs/ARBs or with verapamil/diltizem.[98]

Using two separate drugs with complementary mechanisms of action for the treatment of hypertension has long been accepted by physicians. Fixed-dose combinations of two complementary drugs are gaining acceptance. Such low-dose combination therapy has resulted in better BP control, fewer adverse effects, prolonged duration of the antihypertensive effect due to different half lives of component drugs, lower cost of care and increased patient compliance.[94] Several dose strengths of fixed dose combinations are available which give dosing flexibility.

1.4.1 Combination of β blockers and diuretics

The addition of diuretics has been shown to improve the antihypertensive efficacy of β_1-blockers in African-American patients and other populations with low-renin hypertension. However, both of these drug classes have been shown to have similar adverse effects in that they increase the risk of glucose intolerance, the development of new-onset diabetes, fatigue and sexual dysfunction. Outcome studies have shown a morbidity and mortality reduction with diuretics and β_1-blockers in combination.[10]

Combinations listed in JNC VII are atenolol/chlorthalidone, bisoprolol fumarate/ HCTZ, propranolol LA (long acting)/HCTZ, metoprolol tartrate/HCTZ, nadolol/ bendro-flumethiazide and timolol maleate/HCTZ.[23]

1.4.2 Diuretic combinations

The JNC VII-reported diuretic combinations are amiloride HCl/HCTZ, spironolactone/HCTZ, triamterene/HCTZ.[23]

Combination therapy has been attempted with a potassium-sparing diuretic and a thiazide diuretic to reduce the risk of adverse metabolic effects. Combination therapy does not obviate the need for serial monitoring of serum electrolyte levels, but it does decrease the incidence of thiazide-induced hypokalemia without an increased risk of hyperkalemia.[99]

1.4.3 Targeting CCBs

1.4.3.1 Dual calcium channel blockade

The combination of a dihydropyridine CCB with either verapamil or diltiazem has been shown in a recent metaanalysis to have an additive effect on blood pressure lowering without significantly increasing adverse events. Dual CCB blockade may be useful in patients with documented angioedema on RAS inhibitors or in patients with advanced renal failure at risk for hyperkalaemia. However, no outcome data are available with dual CCB therapy and long-term safety remains undocumented.[100]

1.4.3.2 Combination of CCBs and diuretics

Most physicians are somewhat reluctant to combine a CCB with a diuretic. However, in the VALUE trial, hydrochlorthiazide was added as a second step in patients randomized to amlodipine. The diuretic/CCB combination was found to be well tolerated, although there was a higher risk of new onset diabetes and hyperkalaemia when compared with the valsartan arm.[101] The use of diuretics plus calcium channel blockers for hypertension may be associated with a higher risk of myocardial infarction but not stroke, compared with a combination of diuretics and β blockers.[102]

1.4.4 Targeting RAS

1.4.4.1 RAS and diuretic combination

Data from randomized double-blind placebo-controlled clinical trials have shown that an ARB in combination with hydrochlorothiazide is significantly more efficacious than either of the agents alone and the combination has an excellent adverse event profile. Fixed-dose combinations of an ARB and low-dose hydrochlorothiazide provide a convenient and effective treatment option for patients who do not achieve blood pressure targets on monotherapy, without compromising the placebo-like tolerability of ARBs.

The combinations of ACEIs and diuretics listed in JNC VII are benazepril/HCTZ, captopril/HCTZ, enalapril maleate/HCTZ, lisinopril/HCTZ, moexipril HCl/HCTZ and quinapril HCl/HCTZ.[23] An international randomized controlled trial has shown that antihypertensive therapy using perindopril and indapamide significantly reduces the recurrence of stroke; 62.8% of the patients achieved the blood pressure goal. The incidence of adverse events was significantly higher in the combination therapy group than in the perindopril monotherapy group. If adequate care of compromised renal function is taken, perindopril plus diuretic combination therapy exerts potent hypotensive effects without posing significant safety problems in patients with a history of stroke.[103]

1.4.4.2 Combination of ARBs and ACEIs

Current treatment regimens with ACEIs and ARBs may not completely suppress

the RAS. Combinations of ACEIs and ARBs have been shown to be superior to either of the agents alone for some, but certainly not for all composite cardiovascular and kidney

Table 3. Effect of combination therapy with ACEIs and ARBs

Study	Patient No.	Patient Characteristics	Results	Outcome
ValHeFT[38]	5,010	Class II–IV CHF	Addition of valsartan to ACE inhibitor was superior to placebo in lowering BP	Addition of valsartan to ACE inhibitor significantly decreased mortality and morbidity
RESOLVD [106]	426	Class II–IV CHF	Trend toward lower systolic BP with candesartan plus enalapril	Combination of candesartan plus enalapril was significantly superior to either drug alone in improving cardiac ejection fraction
CHARM-added [107]	2,548	Class II–IV CHF and ejection fraction ≤40%	Addition of candesartan to ACE inhibitor resulted in significantly greater BP reductions than addition of placebo	Combination of candesartan plus an ACE inhibitor significantly decreased risk versus placebo plus an ACE inhibitor for primary composite outcome of cardiovascular death or hospitalization for HF
CHARM-overall [108]	7,601	Class II–IV CHF and ejection fraction ≤40%	Not reported	Combination of candesartan plus an ACE inhibitor significantly decreased all-cause mortality
Cice [109]	80	CHF and ejection fraction <40%	Not reported	Addition of telmisartan to ACE inhibitor significantly decreased the risks for CHF hospitalization, all-cause mortality, and cardiovascular death
VALIANT [37]	4,909	Recent myocardial infarction	Addition of valsartan to captopril was significantly superior to placebo in lowering BP	No significant differences between treatment groups with regard to the primary outcome measure, death from any cause
CALM [110]	199	Diabetes mellitus, hypertension, and proteinuria	Combination of lisinopril and candesartan was significantly superior to either drug alone in lowering BP	Combination of lisinopril and candesartan was significantly superior to either drug alone in decreasing the albumin/ creatinine ratio
CALM II [111]	75	Diabetes mellitus plus hypertension	Combination of candesartan and low-dose lisinopril was not significantly superior to high-dose lisinopril in lowering BP	Combination of candesartan and lowdose lisinopril was not significantly superior to high-dose lisinopril placebo in lowering the albumin/ creatinine ratio
COOPERATE [112]	263	Chronic non-diabetic nephropathy	Reductions in BP similar to losartan, trandolapril, and the combination	Losartan plus trandolapril was significantly superior to either drug alone for decreasing risk for the composite endpoint of doubling of serum creatinine level or progression to end-stage renal disease

outcomes. The RAS blockade with ACEIs and ARBs has antihypertensive and pleiotropic effects conferring cerebral, cardiac and renal target-organ protection. In clinical trials, ACEIs and ARBs have demonstrated reno- and cardioprotection (**Table 3**).[104, 105]

1.4.5 Combination of RAS inhibitors and CCBs

CCBs have been shown to be amenable to combination with other antihypertensive drugs including ARBs and ACE inhibitors. The additive effect observed with combination therapy most likely occurs because of differing modes of action providing synergistic or complementary effects.

Complementary action of dihydropyridine CCBs with ARBs results from arteriolar dilation and natriuresis by the CCB and counteraction of the effects of stimulated angiotensin II by the ARBs. Another benefit of this combination is the alleviation of pedal edema associated with dihydropyridine CCB monotherapy.[113-116] The first approved ARB-CCB combination of valsartan and amlodipine was supported by a study[113] that evaluated the efficacy and safety of different amlodipine and valsartan dose combinations over an 8-week period in 1911 patients with hypertension. The next approved ARB/CCB combination, olmesartan medoxomil and amlodipine, was supported in a study by Chrysant et al[114] that evaluated the efficacy and safety of different amlodipine and olmesartan medoxomil dose combinations over an 8-week period in 1940 patients with hypertension. The third ARB/CCB combination, telmisartan and amlodipine, was investigated in a study by Littlejohn et al.[117] that evaluated the efficacy and safety of different amlodipine and telmisartan dose combinations over an 8-week period in 1461 patients with hypertension.[118]

Regarding the combination of dihydropyridine **CCBs with ACEIs**, some of the combinations listed in JNC VII are amlodipine/benazepril hydrochloride, enalapril maleate/felodipine and trandolapril/verapamil.[23] Amlodipine/benazepril combinations were well tolerated and resulted in significant BP reductions and better BP responder

rates than amlodipine monotherapy. Addition of benazepril at high doses to amlodipine monotherapy significantly reduced office and ambulatory BP, and increased the BP responder rate. The results of the study suggest that high dose amlodipine/benazepril combination therapy is an effective, safe and well-tolerated treatment option for hypertensive patients who do not respond adequately to amlodipine alone or who have experienced unacceptable edema.[119]

In conclusion, the combination of RAS inhibitors with DHP-CCBs may provide more intensive BP control to currently recommended targets and cardiovascular protective effects that lead to more global risk-factor reduction in patients with hypertension. Given their excellent and complementary tolerability profiles, the combination therapy of an ARB or ACEI with a long-acting DHP-CCB is a rational choice for patients requiring two or more antihypertensive agents.[120]

Now a days the combinations of ARBs, CCBs and diuretic are in the market. Amlodipine-valsartan-hydrochlorothiazide (approved in 2009) is a fixed dose combination of the well established antihypertensive agents. In patients with moderate or severe hypertension, triple combination therapy with amlodipine, valsartan and HCTZ produced significantly greater reductions from baseline in mean sitting systolic and diastolic BP than the combinations of either valsartan and HCTZ, amlodipine and HCTZ or amlodipine and valsartan in a large 8-week randomized double-blind multinational phase III trial.[121]

In several clinical trials, α_1 blockers were allowed or were specified as add-on therapy. Usefulness of the α_1 blocker doxazosin as a third-line antihypertensive drug has been checked with combination of CCBs and ARBs/ACEIs. Results suggest that addition of a low dose of the α_1 blocker doxazosin effectively reduces BP in patients.[122] In the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT) nine patients received extended-release doxazosin as a third drug if they did not reach their goal blood pressure with either the combination of amlodipine plus perindopril or atenolol plus

bendroflumethiazide. It suggested doxazosin as safe and effective addition.[123] The review of reported clinical trials of doxazosin in different groups of hypertensive patients such as diabetics, the elderly, patients with benign prostatic hyperplasia or hypercholesterolaemia, the obese or Afro-Americans and in combination with all major groups of antihypertensive drugs such as CCBs, diuretics, β_1 receptor antagonists, ACEIs and ARBs, doxazosin showed its efficiency. A large proportion of patients demonstrated a favorable blood pressure response with relatively few treatment-associated side effects showing that this drug appears to be a valuable add-on antihypertensive treatment option.[124]

1.4.6 Other combinations

Although aldosterone is a product of the renin-angiotensin system, its production is not inhibited by treatment with either ACEIs or ARBs.[125, 126] This phenomenon is known as aldosterone escape. Combining of ACEIs/ARBs and ARA has been suggested to provide substantial inhibition of entire RAS.

Soluble guanlyate cyclase activators caused vasodilation which might be countered by the effects of reflex up-regulation of the RAS. ACEIs can inhibit this reflex. This synergy may be more than an additive acute effect or a reduced propensity to the development of tolerance following repeated dosing.[127] Prostacyclin analogues were found to be effective in combination with CCBs in the treatment of pulmonary hypertension.[22]Agents interrupting RAS like ARBs and ACEIs are reported to be used in combination.

1.5 Development of multitargeted ligands

Treatment with a fixed dose drug combination (FDC) is a good option as two or more drugs can be co-formulated in a single dosage form simplifying dosing regimens and thereby improving patient compliance[128, 129] However, complications may arise due to highly complex PK/PD relationships of the drugs requiring sophisticated formulations.

Potential drug-drug interactions could have a significant impact on the risks and costs of developing FDCs[130]

An alternative strategy with a different risk - benefit profile is to develop a single chemical entity capable of modulating multiple biological targets simultaneously[131]. A lower risk of drug-drug interactions in comparison to cocktails or fixed drug combinations is a clear advantage of this strategy. Although the development of such multiple-acting ligands can be challenging due to increased complexity in the design and optimization of such ligands, these difficulties are associated with an early and therefore less expensive stage of the drug discovery process. The risks and costs of developing multiple targeted ligands are in principle no different to the development of any other single entity. A number of clinically used drugs have been found to have activity at more than one target, which in some cases is associated with increased efficacy, in others with side effects. In most cases these are historical drugs for which the multiple activity profile was not designed but serendipitously discovered. The rational design of ligands, that act selectively on specific multiple targets of therapeutic interest termed Designed Multiple Ligands (DML), is a more recent trend.

1.5.1 Lead Generation Strategies

Two fundamentally different methods for discovering DML lead compounds have been reported in the literature: *screening* approach and *knowledge-based* approach that exploit information either from the general literature or from proprietary sources.

1.5.1.1 Screening approach

The screening of compound libraries appears to be the most commonly reported approach to DML lead generation (Fig. 2). Interestingly, the predominant screening strategy so far reported is focussed screening rather than high throughput screening (HTS). This helps to simplify the logistics of screening against multiple targets and improves screening hit rates. In focussed screening, compound classes that are already

known to be active against one of the targets of interest are screened against another target. This is a particularly favoured strategy for kinase targets where DMLs are usually

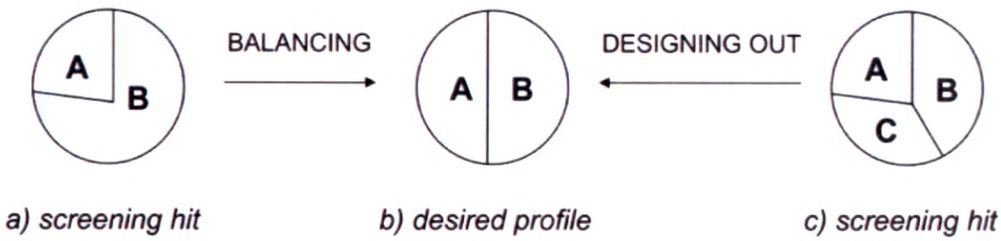


Figure 2: Screening approaches

discovered through the cross-screening of ligands from selective kinase programmes against other kinases. Although DML lead compounds produced by either of these screening approaches would normally have all desired biological activities, it is highly unlikely they would have the desired activity profile. Leads often require "*balancing*", since one of the biological activities would need a greater improvement during the optimization in order to achieve the desired DML profile. In addition to the desired activities, screening hits frequently bind to other targets. To minimize the risk of side effects these undesired activities will need to be "designed out".

1.5.1.2 Knowledge-based approach

The knowledge-based approach, also referred to as *framework combination*, is another lead generation strategy frequently reported in the literature (**Fig. 3**). This approach is based on a combination of frameworks and the underlying pharmacophores of two molecules, each selective for different target of interest into a single molecule to "design in" both activities. The resulting DMLs are termed *linked, fused or merged,* depending upon the extent to which frameworks of the selective ligands have been integrated (**Fig. 3**). At one end of the whole spectrum of possible degrees of integration reported in the literature are linked DMLs, or conjugates, whose molecular frameworks

are in fact not integrated but connected through a distinct linker group not found in either of the starting selective ligands. In some cases linked DMLs contain a metabolically cleavable linker designed to release two ligands *in vivo* that would then interact independently with each target. This could be seen as a half-way scenario between a true DML and a fixed dose combination. However, in most cases the linker is intended to be metabolically stable yielding a single compound capable of interacting with both targets,

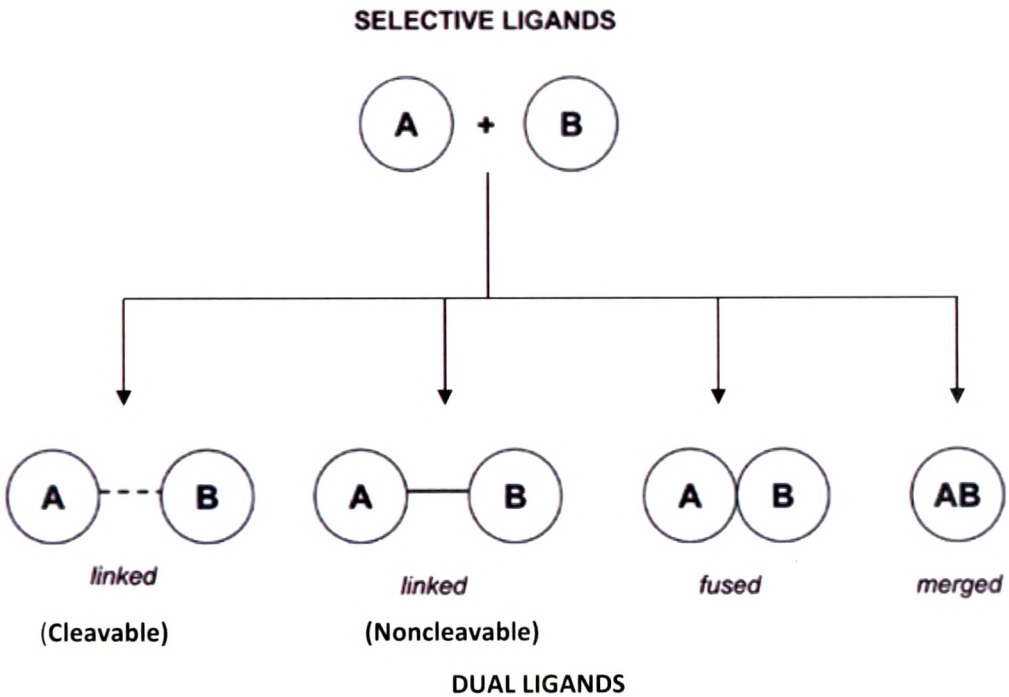


Figure 3: Knowledge-based approaches

albeit different ends of the molecule may be responsible for the activity at the different targets.[132, 133] Medicinal chemists generally aspire to maximize the degree of framework overlap in order to produce smaller and simpler molecules with favorable physicochemical properties. Hence, the most common and most sought after are *merged* DMLs, where the frameworks are integrated by taking advantage of commonalities in the structures of the starting compounds. The screening and knowledge-based approaches can be viewed as complementary strategies. One of the main advantages of the

framework combination approach is a potentially rapid access to a DML starting point, which can be greatly assisted by leveraging the in-depth structure-activity relationship (SAR) knowledge from historical selective ligand projects. Over the recent years efforts have been made to synthesize agents which modulate multiple biological targets simultaneously.[131, 134, 135]

1.5.2 Dual α_1 and β_1 antagonists

Dual inhibition of α_1 and β_1 receptors was considered beneficial as it can decrease pheripheral resistance and cardiac output. The dual acting α and β-blockers may be useful in the management of hypertension. Some dual acting blockers are adimolol, bucindolol, carvedilol, labetalol, medroxalol and primidolol. In experimental studies and in patients with diabetes and hypertension, carvedilol has demonstrated improvements in endothelial vasodilatory and anti-inflammatory functions and in platelet antiaggregation activity.[136] In the GEMINI trial, patients on carvedilol also showed improved insulin resistance and reduced progression to microalbuminuria.[137] Carvedilol helps to produce a desirable hemodynamic profile and facilitates appropriate blood pressure and heart rate responses to exercise. Carvedilol does not appear to adversely affect left ventricular systolic function and in selected patients with heart failure, has been shown to increase the ejection fraction in elderly patients.[138]

1.5.3 β-Blockers with NO vasodilator/β_2-stimulants

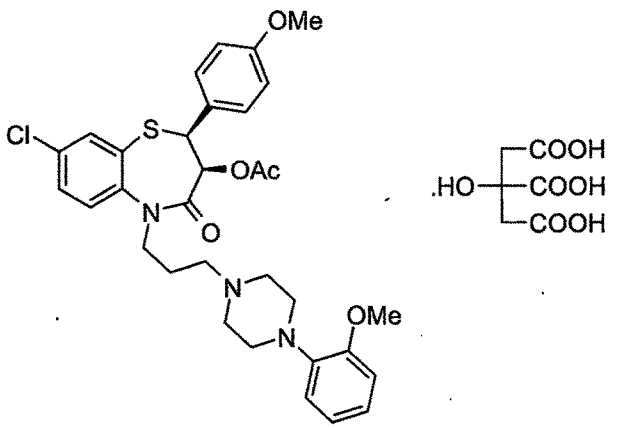
The third generation β_1 blockers are used for the treatment of hypertensive patients, especially with diastolic or systolic dysfunction. Nevibolol (approved in 2007), a highly selective β_1 blocker with an NO-mediated vasodilating effect, looks promising in controlling hypertension[139-142] because it acts by dual mechanisms.[143] The vasodilator effect of nevibolol on the renal artery involves 1) activation of the endothelial β_2-adrenoceptor, 2) participation of Ca^{2+}, 3) increase in NO (by preventing its oxidative degradation) and eNO_S, and 4) activation of Ca^{2+}-activated K^+ channels.[144] SENIORS was conducted to observe the effects of nevibolol in older patients with heart failure

independent of LV ejection fraction (LVEF). A primary outcome showed nevibolol group representing a significant 14% relative risk ratio compared to the placebo group.[145]

Celiprolol, a cardioselective β-blocker with a stimulant effect on β_2 receptors, is as effective an antihypertensive agent as other β-blockers.[146, 147] Celiprolol is useful in treating hypercholesterolemic hypertensive patients because it improves lipid profile (decrease in total cholesterol, low density lipoprotein cholesterol and triglycerides, and increase in high density lipoprotein cholesterol).[148]

1.5.4 Dual CCB and α_1 antagonists

S-2150 (**I**) also inhibited [^{3}H] WB4101 binding to rat cerebral cortical membrane with a mean K_i value of 0.021 μM. It produced relaxation with an IC_{50} of 190 nM in rat



(**I**)

thoracic aorta rings without endothelium, precontracted with KCl (18 mM). S-2150 (**I**) exerted a clear hypotensive effect in spontaneously hypertensive rats (SHR), two-kidney one-clip renal hypertensive rats (RHR) and normotensive rats (NR).[149]

1.5.5 Dual RAS and neutral endopeptidase (NEP) inhibitors

The physiologic interaction of the renin-angiotensin, the kallikrein-kinin and the natriuretic peptide systems in the regulation of body fluid volume and arterial blood pressure provide a rationale for simultaneously modulating these systems in the treatment of disorders such as hypertension and congestive heart failure. The RAS, kallikrein-kinin

system and the natriuretic peptides are important modulators of cardiovascular homeostasis. These systems alter in conditions such as hypertension and CHF, leading to the rationale of simultaneously blocking these systems.[150]

The dual inhibition of AT_1 and NEP could provide clinical benefits in a range of cardiovascular diseases including hypertension and heart failure. LCZ696 (Novartis; East Hanover, NJ, USA) is a dual acting ARB and neprilysin inhibitor. Treatment with LCZ696 provided significant reductions in blood pressure compared to valsartan. This shows that dual inhibition of the ang II receptor and neprilysin have complementary effects.[151] LCZ696 is in the phase II of development. There are two more molecules namely daglutril (phase II) and VNP489 (phase I) which are based on the same concept.[77] In Ruilope study, patients with mild to moderate hypertension were effectively treated by LCZ696 compared to placebo.[152]

1.5.5.1 Dual vasopeptidase (ACE and NEP) inhibitors

Combined inhibition of NEP and ACE produces cardiovascular effects greater than those elicited by selective inhibition of either of the enzymes alone. Moreover, renin-angiotensin, the kallikrein-kinin and the natriuretic peptide systems, all converge at two key regulatory enzymes which are now known to have structurally similar active sites, ACE and NEP. The development of dual metalloprotease inhibitors (**Table 4**), which inhibit both ACE and NEP, exploit this fortuitous complementarity between the active sites and the physiologic roles of these two enzymes and provides a novel approach to the treatment of cardiovascular diseases[153-158] The simultaneous inhibition of both NEP and ACE in animal models of hypertension and heart failure produces hemodynamic or renal effects which are more than additive when compared with those caused by inhibition of either one of these enzymes alone[159-164]

Early studies with vasopeptidase inhibitors were encouraging. Omapatrilat reduced blood pressure in stroke-prone spontaneously hypertensive rats[165] and salt sensitive rats[166] as well as in individuals with mild to moderate hypertension.[167] Larger

trials of omapatrilat, such as OVERTURE,[168] and OCTAVE,[169] confirmed that combined ACE and NEP inhibition might be effective in the treatment of hypertension and heart failure, but also validated concerns about the higher incidence of angioedema with combined therapy than with ACE inhibition alone. Clinically omapatrilat produced great-

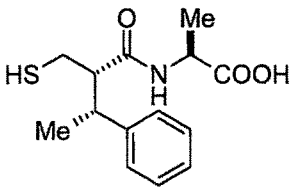
Table 4. Selected vasopeptidase inhibitors[77, 154]

Sr. No.	Compound	Company	Phase of development
1	Omapatrilat	Bristol- Mayers squibb	Phase III
2	Sampatrilat	Roberts	Phase II
3	Gemopatrilat	Bristol- Mayers squibb	Phase I/II
4	MDL-100240	Aventis	Phase II/III
5	Fasidopril	Eli Lilly	Phase II
6	Z-13752A	Zambon/Glaxo Smithkline	Phase II

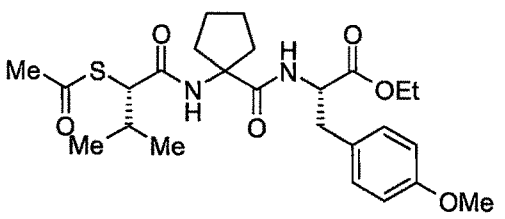
er reductions in peripheral and central pulse pressure in association with a pressure-independent reduction in proximal aortic stiffness. These findings are consistent with a favorable effect of natriuretic peptides on central conduit vessel function.[170] Sampatrilat was shown to lower blood pressure in patients with hypertension.[171] Some researchers have reported molecules which possessed dual antagonism as discussed below.

S21402 (**II**) is a sulfhydryl-containing inhibitor of both NEP (K_i = 1.7 nM) and ACE (K_i = 4.2 nM). S21402 has been tested with purified rabbit kidney NEP and with mouse lung membrane as a source of ACE. The K_i value for NEP is 1.7 nM with ^{3}H-D-Ala-Leu enkephalin as a substrate, and the K_i for ACE is 4.8 nM with NCbz-Phe-His-Leu as a substrate. Oral S21402 reduces systolic blood pressure in an ACE inhibition-sensitive model (SHR) and in a NEP inhibition-sensitive model (DOCA-salt rats).[172]

CGS 30440 (**III**) is a thioacetyl-containing dipeptide, which is believed to be metabolized *in vivo* to its biologically active form CGS 30008 (**IV**). CGS 30440 (**III**) had IC_{50} values of 19 nM for ACE and 2.2 nM for NEP. CGS 30440 (**III**) blocked ang I pres-



(**II**)



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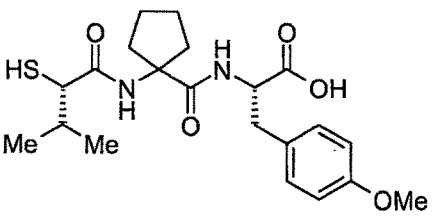


(**IV**)

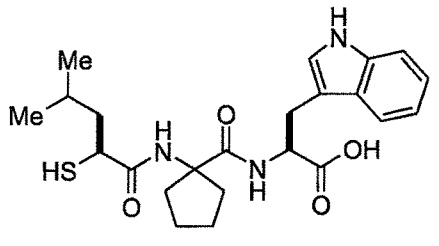
sor responses and increased plasma ANP immunoreactivity during the infusion of exogenous ANP to Sprague-Dawley rats. *In vivo,* CGS 30440 reduced plasma and lung ACE activity and kidney NEP activity in Sprague-Dawley rats for 24 h following a single administration.[173]

1.5.5.2 Triple vasopeptidase inhibitors

One potential limitation of the ACE/NEP dual inhibition approach is an increase in plasma levels of endothelin 1 (ET-1), a vasoconstricting peptide similar to ang II that is degraded by NEP. This might be overcome by additionally inhibiting endothelin converting enzyme (ECE-1). CGS 35601 (**V**), a triple vasopeptidase inhibitor (VPI), may represent a novel class of antihypertensive drugs and may have the potential to reduce morbidity and mortality from cardiovascular disorders, diabetes and subsequent renal complications. CGS 35601 (**V**) is one of a few single molecules capable of inhibiting the activities of ACE, NEP and ECE simultaneously, with IC_{50} values of 22, 2 and 55 nM, respectively. In order to improve the oral bioavailability of CGS 35601, the S-acetyl,

methyl ester prodrug CGS 37808 (**VI**) was synthesized. At an oral dose of 10 mg Eq/kg, it inhibited the ang I-induced pressor response by an average of 49% for 4 h and potentiated the plasma ANP levels by 103% when compared with vehicle-treated rats.[174]

Researchers reported a series of compounds for triple inhibition of ACE, NEP and ECE-1. One of the best compounds derived from this approach was the indanyl analogue (**VII**) displaying binding affinity toward ACE, NEP and ECE-1 with 1.3, 24 and 10 nM respectively.[175]



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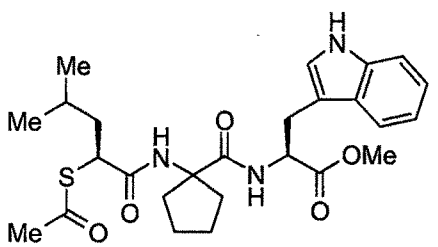

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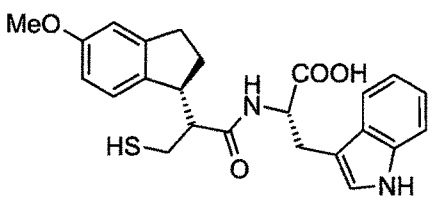


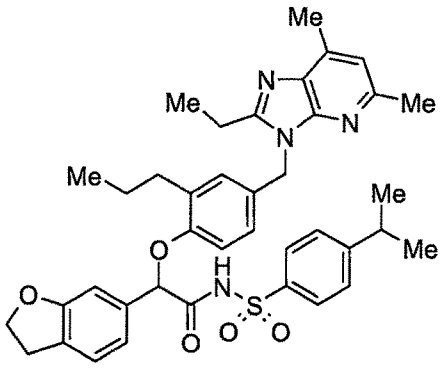
(VII)

1.5.6 Miscellaneous

1.5.6.1 Dual ARB and endothelin receptor antagonists

A combination of the AT_1 selective antagonist losartan and the ET_A/ET_B selective antagonist SB-290670 produced an additive reduction in blood pressure compared to either of the drugs alone, prompting groups at Merck and BMS to develop simultaneous blockers of AT_1 and ET_A receptors. Merck and BMS worked on to develop simultaneous blockers of AT_1 and ET_A receptors that resulted into compound (**VIII**) which exhibited balanced activity at all four receptors (AT_1- 0.013, AT_2 -0.032, ET_A- 0.024 and ET_B- 0.06 uM).[176] Another work carried out using the same strategy resulted into balanced antagonist (**IX**) with binding affinity of 0.8 and 9.3 nM for AT_1 and ET_A

respectively.[177] PS433540 (Pharmacopeia) is in the phase II of development possessing dual antagonism of AT_1 and ET_A. The investigators reported PS433540 to be safe and well tolerated.[178]



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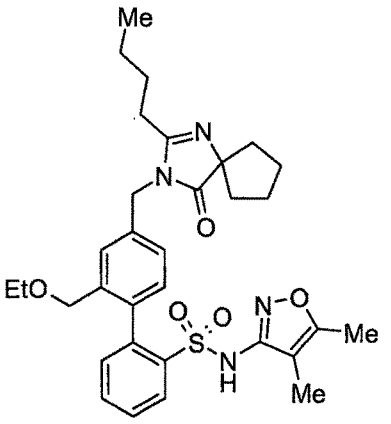
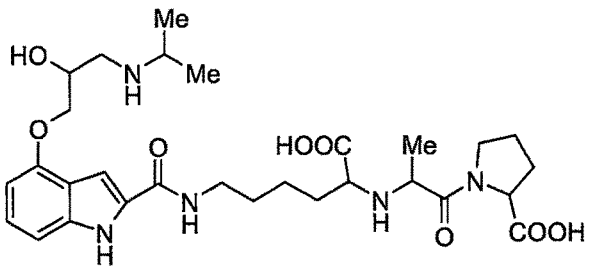


(IX)

1.5.6.2 Dual ACE and β receptor antagonists

BW A385C (**X**) originated from a programme of research with the objective of developing a novel hybrid drug incorporating both ACE inhibitory and β-receptor blocking properties. The agent produces a competitive blockade of heart rate responses to isoprenaline in a guinea pig right atrial preparation with a pK_b of 6.7 (β receptor blocking properties) and (IC_{50}) of 1.2 ± 0.18 nM (ACE inhibition). *In vitro* and *in vivo*



(X)

studies have shown that BW A385C possesses both ACE inhibitory and β receptor blocking properties. BW A385C reduces blood pressure, after acute administration without elevating heart rate and without compromising either cardiac or renal function.[179]

1.5.6.3 Dual ARB antagonists and PPARγ agonists

Telmisartan was later on found to be a multitargeted ligand. ARBs possess partial agonism of peroxisome proliferator-activated receptor gamma (PPARγ) receptor. Data provides a novel insight that telmisartan inhibits AT_1 receptor gene expression through PPARγ activation. The dual inhibition of ang II function by telmisartan – AT_1 receptor blockade and its downregulation – would contribute to more complete inhibition of the RAS. Telmisartan, an ARB and a partial agonist of PPARγ, may be quite useful for the treatment of patients with hypertension with complications such as diabetes and atherosclerosis.[180] A DML may be more useful for microalbuminuria reduction than ARBs with no PPARγ agonistic action. Telmisartan achieved more microalbuminuria reduction than an ARB with no PPARγ agonistic action, possibly through suppression of the inflammatory state in metabolic hypertensive patients.[181] Two more molecules azilsartan and PF-03838135 are reported to possess AT_1 receptor antagonism and a partial agonism of PPARγ.[77]