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Review

# New drug targets for hypertension: A literature review

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ABSTRACT

Hypertension is one of the most prevalent cardiovascular diseases worldwide. However, in the population of resistant hypertension, blood pressure is difficult to control effectively. Moreover, antihypertensive drugs may have adverse effect currently. Hence, new therapeutic targets and treatments are needed to uncovered and exploited to control hypertension and its comorbidities. In the past, classical drug targets, such as the aldosterone receptor, aldosterone synthase, and ACE2/angiotensin 1–7/Mas receptor axis, have been investigated. Recently, vaccines and drugs targeting the gastrointestinal microbiome, which represent drug classes, have also been investigated for the management of blood pressure. In this review, we summarized current knowledge on classical and new drug targets and discussed the potential utility of new drugs in the treatment of hypertension.

## 1. Introduction

Hypertension is a major contributing factor for cardiovascular disease (CVD) and renal diseases, that can increase the risks of comorbidities such as myocardial infarction, stroke and heart failure (HF) [1]. Studies have revealed that risk factors such as obesity and genetic factors can influence the occurrence and development of hypertension [2,3]. In addition, complicated regulatory networks, including the renin-angiotensin-aldosterone system (RAAS), the nervous system and arterial remodeling [4–6], also affect the progression of hypertension. Because blood pressure (BP) is difficult to control, the priority is finding drug targets to effectively control and manage BP in the hypertensive population. In this review, we primarily describe the classical and new drug targets used in hypertension therapy.

## 2. Classical targets in hypertension

Renin-angiotensin-aldosterone system (RAAS) plays an important role in human body. The imbalance of RAAS could result in the occurrence of the hypertension directly. Briefly, Renin (or angiotensinogenase) secreted by kidney catalyzes its substrate angiotensin which is the other component in RAAS system synthesized by liver, contributing to form angiotensin II accompanying with the effect of the angiotensin-converting enzyme (ACE). Meanwhile, aldosterone secreted by adrenal gland could maintain sodium-potassium homeostasis by increasing sodium reabsorption with mineralocorticoid receptor (MR). Hence, the component of RAAS system can serve as therapeutic targets to regulate blood pressure and many pharmacological antihypertensive drugs were developed on the basis of this (Fig. 1). Classical antihypertensive drugs include renin inhibitor, ACE inhibitors, angiotensin II receptor blockers

*Abbreviations:* AR, aldosterone receptor; MR, mineralocorticoid receptor; CVD, cardiovascular disease; MRA, mineralocorticoid receptor antagonist; ACE, angiotensin-converting enzyme; RAAS, renin-angiotensin-aldosterone system; ARTS, antagonist tolerance study; CKD, chronic kidney disease; SHR, spontaneously hypertensive rat; MTD, maximum tolerated dose; MABP, mean arterial blood pressure; DOCA, deoxycorticosterone acetate; WKY, Wistar-Kyoto; AT1R, ang type 1 receptor; AT2R, ang type 2 receptor; C-21, Compound 21; RPT, renal proximal tubule; NHE3, $Na^+/H^+$ exchanger 3; NKA, $Na^+/K^+$ ATPase; NO, nitric oxide; APA, aminopeptidase A; RAS, renin-angiotensin system; BP, blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; ETR, endothelin receptor; ETRA, endothelin receptor antagonist; NOS, NO synthase; ADMA, asymmetric dimethylarginine; SDMA, symmetric dimethylarginine; DEX, dexamethasone; NAC, *N*-acetylcysteine; HF, heart failure; VIP, vasoactive intestinal peptide; VLP, virus-like particle; SCFA, short-chain fatty acid.

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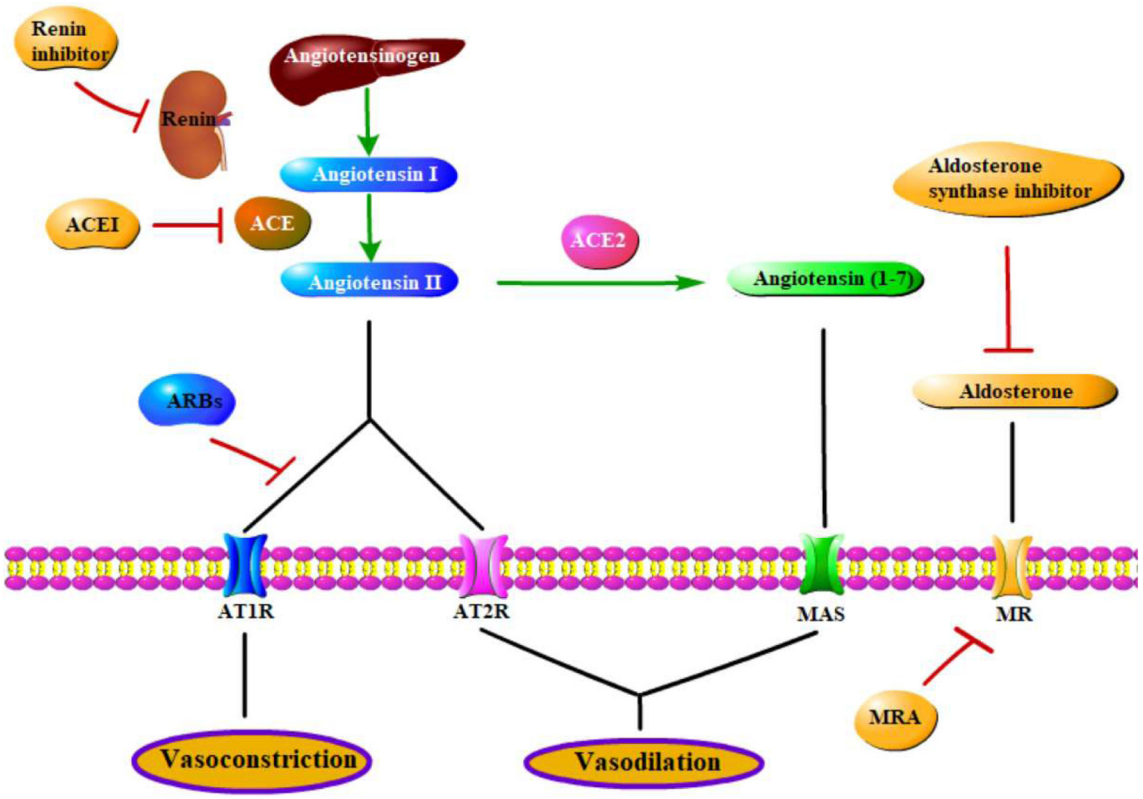


**Fig. 1.** Classical targets in renin-angiotensin-aldosterone system.

(blocking the activity of Ang1–7/Mas and Ang II-Ang type 1 receptor (AT1R)/Ang type 2 receptor (AT2R)), $\beta$-adrenoreceptor blockers (blocking the secretion of renin), aldosterone-related blocker (blocking the activity of the synthesis of aldosterone and receptor).

Here, we will review some classical targets and their roles in RAAS system.

### 2.1. AngII-AT1R/AT2R axis

Ang II mainly works by activating AT1Rs and AT2Rs. AT1Rs mediate vascular smooth muscle contraction, aldosterone secretion, dipsogenic responses, renal sodium reabsorption and pressor and tachycardiac responses [23]. Conversely, AT2Rs generally induce the opposite effects, including vasodilation, natriuresis, cellular differentiation and growth inhibition [24]. Therefore, AT2R agonists could serve as a potential therapeutic drug for the treatment of hypertension. Compound 21 (C-21) is a highly selective nonpeptide AT2R agonist which is the first reported AT2R agonist [25]. C-21-induced AT2R activation evoked a bradykinin-nitric oxide (NO)-cyclic guanosine 3,5-monophosphate (cGMP) signaling cascade that stimulated the downstream signaling mediators Src kinase and extracellular signal-related kinase, leading to the internalization/inactivation of the major renal proximal tubule (RPT) $Na^+$ transporters $Na^+/H^+$ exchanger 3 (NHE3) and $Na^+/K^+$ ATPase (NKA) and resulting in natriuresis [26]. Prior studies found that Ang II can increase sodium retention and BP in rats, however, the injection of C-21 prevented Ang II-mediated sodium retention and BP elevation. The activation of chronic AT2R initiates and sustains receptor translocation to RPT apical plasma membranes. It also promotes the internalization/inactivation of NHE3 and NKA and prevents $Na^+$ retention, which results in a negative cumulative $Na^+$ balance and lowers BP in models of experimental Ang II-induced hypertension [24]. The results indicated that C-21 is a potential drug candidate for the treatment of hypertension and $Na^+$ retaining states in humans. In addition, in the clipped kidneys of two-kidney, one-clip hypertensive rats model, C-21 significantly reduced TNF-α, IL-6 and TGF-β1 levels and increased nitric oxide (NO) and cGMP levels in the kidneys [27]. These results suggest that AT2R is a new target for the treatment of hypertension and AT2R agonists may act as novel anti-hypertensive drugs in the future.

### 2.2. ACE2/Ang1–7/Mas receptor axis

In addition to the classic ACE-Ang II-ATR1 axis, the RAAS system also features the ACE2/Ang1–7/Mas axis. In contrast to the ACE-Ang II-ATR1 axis, the ACE2/Ang1–7/Mas axis inhibits ventricular remodeling and lowers BP by inducing systemic and regional vasodilation, promoting diuresis and natriuresis and inhibiting the proliferation and migration of smooth muscle cells, cardiomyocytes, fibroblasts and glomerular and adjacent tubular cells [68]. ACE2 is similar to ACE structurally. It can generate Ang1–9 by cleaving Ang I and Ang1–7 can be generated from Ang1–9 through the action of ACE. It can also directly cleave Ang II into Ang1–7 [69]. Ang1–7 participates in vasodilation, natriuresis and BP reduction by binding to the MAS receptor. Therefore, drugs targeting ACE2 or MAS may be useful in the treatment of hypertension. AVE 0991, a MAS receptor agonist, can reduce mean arterial BP (MABP) in rats with hypertension induced by deoxycorticosterone acetate (DOCA). When combined with renin inhibitors, the anti-hypertensive effect is stronger [70]. Moreover, AVE 0991 can reduce BP and cardiac inflammatory cell infiltration and collagen fiber deposition in renovascular hypertensive rats and it plays a role in reducing BP-induced cardiac remodeling and improving baroreflex sensitivity [71].

A study [72] involving 161 patients with essential hypertension and 47 age- and sex-matched normotensive healthy subjects revealed that plasma ACE2 levels were significantly elevated in patients with hypertension, compared with healthy individuals. Additionally, the concentration of ACE2 in serum was positively correlated with left atrial diameter, left ventricular end-diastolic diameter and left ventricular

mass in patients with hypertension. In addition, a prospective controlled study found that the expression of ACE2 was increased in patients with acute ST-elevation myocardial infarction and ACE2 activity in plasma was closely related to the infarct area, left ventricular systolic dysfunction and the occurrence of left ventricular remodeling [73]. These results suggest that ACE2 may participate in the development of hypertension and cardiovascular disease. Human and murine recombinant ACE2 are pharmacological tools for strenthening the activity of ACE2. A study illustrated that compared with the results in the control group, mice with Ang II-induced hypertension that were injected with murine recombinant ACE2 exhibited lower BP pressure [74]. Previous research indicated that in mice infused with Ang II (1.5 mg/kg/day) for 4 days, Ang II levels and nicotinamide adenine dinucleotide phosphate oxidase activity were higher in ACE2-knockout mice than in wild-type mice. Meanwhile, pro-inflammatory cytokine and fibrosis-associated gene levels were also higher in ACE2-knockout mice than in wild-type mice. Then, the mice were treated with recombinant human ACE2 (2 mg/kg/day, intraperitoneal), and the results confirmed that recombinant human ACE2 prevents Ang II-induced hypertension, renal oxidative stress and tubulointerstitial fibrosis [75]. These results suggest that BP can be reduced by enhancing ACE2 activity. In prior studies, the ACE2 activator XNT (1-[(2-dimethylamino)ethylamino]-4-(hydroxymethyl)-7-[(4-methylphenyl)sulfonyloxy]-9H-xanthene-9-one) was found to lower BP in SHRs and Wistar-Kyoto (WKY) rats, as well as improving the heart function of SHRs and reverse myocardial, peripheral vascular and renal fibrosis [76]. In addition, XNT reduced BP in Ang II-induced hypertensive, wild-type and ACE2-knockout mice [77][]. All of these studies indicate that the development of drugs targeting the ACE2/Ang1–7/Mas receptor axis will be of great significance for the treatment of hypertension and the reduction of heart and kidney fibrosis.

### 2.3. ACE2 in COVID-19

Recently, it is a widespread idea that ACE2, an important component in RAAS system is the cell membrane receptor of SARS-Cov-2, the causative virus of the COVID-19 pandemic, which emerged from Wuhan, China in 2019 [28]. The entry of SARS-CoV-2 into host cell is mediated by a spike protein which is a special region in SARS-Cov-2. The binding of spike protein with ACE2 and its “accomplice” protein TMPRSS2 leads SARS-Cov-2 to host cell, increasing the secretion of inflammatory cytokine and causing the consequent inflammation storm in lung [29,30]. Recent studies showed that SARS-CoV-2 can directly infect engineered human blood vessel organoids and human kidney organoids, which can be inhibited by human recombinant soluble ACE2 (hrsACE2) [31]. Moreover, research showed that circulating Ang II levels were markedly elevated compared with healthy control [28], and overexpression of the Fc domain of spike protein elevated ANG II levels which indicated that imbalance of ACE2 and ANG II existed in the RAAS.

Based on that, many classical antihypertensive drugs including angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin II receptor blockers (ARBs) were applied to treat the COVID-19 patients due to the ability of blocking ACE2 and down-regulating the ANG II [32]. Among the COVID-19 patients with severe symptoms, most of them have pre-existing comorbidity such as hypertension and so on. In one study, among the patients with severe symptoms of COVID-19, 58% of them had hypertension, 25% of them had heart disease and 44% of them had arrhythmia [33]. Hence, it is useful and necessary to treat the patients with antihypertensive agents. A retrospective, single-center study showed that ARBs/ACEIs group had significantly lower concentrations of Hs-CRP and procalcitonin compared with non- ARBs/ACEIs group, and a lower proportion of critical patients and a lower death rate were observed in ARBs/ACEIs group than non-ARBs/ACEIs group, whereas it failed to reach statistical significance [34]. These results revealed that ACE2 played an important role in the development and treatment of COVID-19 besides hypertension.

### 2.4. Aldosterone

The aldosterone synthetase CYP11B2 can catalyze the synthesis of aldosterone [35,36]. CYP11B2 is a key enzyme involved in the final stages of the biosynthesis of aldosterone. Therefore, inhibition of the expression of CYP11B2 is a potential treatment strategy for diseases such as hypertension, congestive HF and myocardial fibrosis [37]. Ménard et al. [38] revealed that the aldosterone synthase inhibitor FAD286A dose-dependently reduced the urine aldosterone concentration in spontaneously hypertensive rats (SHRs) and increased plasma renin concentrations. Furthermore, this drug reversed hypokalemia induced by treatment with the diuretic furosemide. In addition, in uninephrectomized rats treated with Ang II and high-salt diet, FAD286 reduced BP and plasma aldosterone levels. The compound also decreased hypertrophy and interstitial fibrosis of the kidneys and heart and exerted protective effects against end-organ damage [39].

LCI699 was the first aldosterone synthase inhibitor to be used orally in humans [40]. Studies revealed that in transgenic rats harboring the renin and angiotensinogen genes, LCI699 reduced the aldosterone levels in plasma and urine in a dose-dependent manner. LCI699 also reduced heart and kidney dysfunction and prolonged the lifespan of rats. In addition, a randomized double-blind experiment involving 99 healthy subjects revealed that compared with the control group, the levels of aldosterone in plasma and urine were decreased by $49 \pm 3$ and $39 \pm 6\%$ respectively in the 0.5 mg LCI699 group together with increased urinary sodium excretion and plasma renin activity [41]. These results suggest that the inhibition of aldosterone synthase is an effective strategy for treating diseases associated with excess aldosterone. The CLCI699A2201 study designed to investigate the role of LCI699 in patients with essential hypertension indicated that compared with the placebo group, all doses of LCI699 (0.25 mg once daily, 0.5 mg once daily, 1.0 mg once daily and 0.5 mg twice daily) reduced systolic BP (SBP) in the clinic-[42]. In addition, a study of 14 patients with increased primary aldosterone revealed that subjects taking LCI699 had lower plasma aldosterone concentrations and higher 11-deoxycorticosterone and potassium ion concentrations. Most importantly, LCI699 reduced 24-h dynamic SBP by 4.1 mmHg [43]. These results suggest that aldosterone synthase inhibitors can be used to treat hypertension. Because aldosterone synthase (CYP11B2) and cortisol synthase (CYP11B1) have high homology, aldosterone synthase can affect the synthesis of cortisol. In the aforementioned CLCI699A2201 study, LCI699 suppressed adrenocorticotropic hormone-induced cortisol secretion in approximately 20% of the subjects [42]. Amar et al. also revealed that plasma cortisol response to corticotropin was reduced in patients with increased primary aldosterone who took LCI699. These results suggest that LCI699 could inhibit cortisol synthesis in addition to inhibiting aldosterone synthesis [43]. Therefore, it is necessary to explore the maximum tolerated dose (MTD) of LCI699 that does not seriously affect cortisol synthesis. One study [44] explored the MTD of LCI699 in patients with hypertension, indicated that LCI699 can lower BP and increase plasma aldosterone levels in line with previous results. Furthermore, the MTD was obtained using a threshold of an ACTH-stimulated cortisol response of $<400$ nmol/L in no more than 20% of patients and no clinical symptoms of adrenal insufficiency. It was estimated that the MTD was 1.30 mg once daily with a 90% confidence interval (CI) of 0.88–181 mg once daily.

Because the selectivity of LCI699 is not strong, researchers have recently applied a ligand-based approach to synthesize a series of novel pyridyl- or isoquinolinyl-substituted indolines and indoles. Compared with LCI699, these compounds are more selective for CYP11B2 than for CYP11B1 [45]. The novel aldosterone synthase inhibitor BI 689648 [6-(5-methoxymethyl-pyridin-3-yl)-3,4-dihydro-2H-[1,8]naphthyridine-1-carboxylic acid amide] was recently discovered. Results revealed that compared with FAD286 and LCI699, BI 689648 has higher selectivity for aldosterone synthase than cortisol synthetase in vitro. Similarly, BI 689648 was found to be more selective than FAD286 and LCI699 in

rhesus monkeys [46]. Whether these new aldosterone synthase inhibitors can selectively inhibit aldosterone synthesis also requires animal experiments and clinical trials.

### 2.5. Aldosterone receptor (AR)

Aldosterone is a mineralocorticoid secreted by the globular zone of the adrenal cortex [7] that can promote the reabsorption of sodium and chloride ions and increase the excretion of potassium and hydrogen ions in the renal tubules. Aldosterone exerts its effects by binding with the mineralocorticoid receptor (MR) [8]. MR activation in the kidneys promotes the development of hypertension by increasing the expression of potassium channels, thereby promoting the reabsorption of water and sodium and causing the loss of potassium in tissues. In addition, the activation of MR in extrarenal tissues such as the heart and blood vessels increases NADPH oxidase levels and the production of reactive oxygen species and promotes the development of hypertension and CVD [9]. Mineralocorticoid receptor antagonists (MRAs) antagonize the action of aldosterone on MR [10]. The two types of MRAs are classic steroids and new nonsteroidal compounds. Steroidal compounds such as spironolactone and eplerenone inhibit the effects of aldosterone by competitively binding its ligand-binding domain on MR and preventing MR from forming its active structure [11].

Spironolactone was the first reported MRA [12]. Studies have revealed that in patients with resistant hypertension with and without primary aldosteronism, the combination of low-dose spironolactone, an angiotensin (Ang)-converting enzyme (ACE) inhibitor or Ang receptor blocker, and diuretics can reduce BP in patients with resistant hypertension [13]. Chapman et al. [14] also reported similar results. Their study found that spironolactone can effectively lower BP in patients with uncontrolled hypertension. In addition, Pitt et al. [15] demonstrated that the combination of spironolactone with standard therapy can substantially reduce the risks of morbidity and death in patients with severe HF. The aforementioned results illustrated that spironolactone played an important role in the prevention and treatment of resistant hypertension. However, spironolactone non-selectively binds to MR, and it can also antagonize the androgen receptor, thereby causing a variety of sexual adverse events in both men and women [16]. Subsequently, eplerenone, a more selective MRA, was developed. This drug appears to have weaker anti-androgenic effects than spironolactone [17]. Finerenone is an analog of dihydronaphthyridine compound. Dihydropyridines have anti-MRA activity and function as $Ca^{2+}$ channel blockers [18]. Previous studies found that finerenone has stronger cardiorenal protective effects than eplerenone in hypertension-induced HF rats [19]. Meanwhile, hypertension-induced cardiac hypertrophy mouse, finerenone exerted a stronger inhibitory effect on myocardial hypertrophy than eplerenone [20]. Dihydropyridine calcium channel blockers can function as MRAs and inhibit aldosterone-induced MR activation [18]. The drug was proven to be a potent and highly selective nonsteroidal MRA [21].

A mineralocorticoid receptor antagonist tolerance study (ARTS) was designed to assess the safety and tolerability of finerenone in patients with HF and reduced left ventricular ejection fraction associated with mild or moderate chronic kidney disease (CKD). The study illustrated that finerenone has the same effect with spironolactone in reducing ventricular remodeling. In addition, finerenone can reduce the occurrence of hyperkalemia and renal impairment [22]. Currently, two Phase IIB clinical studies of finerenone are ongoing. Two studies aimed to assess the effect of drug in patients with worsening chronic HF and type 2 diabetes mellitus and CKD (ARTS-HF; ClinicalTrials.gov: NCT01807221) and in patients with type 2 diabetes mellitus and diabetic nephropathy (ARTS-DN; ClinicalTrials.gov: NCT01874431), separately. The aforementioned results demonstrated that antihypertensive drugs targeting AR have good clinical effects, but these drugs are limited by shortcomings such as drug resistance and side effects. It is necessary to develop new anti-hypertensive drugs targeting AR.

## 3. New targets in hypertension

### 3.1. Aminopeptidase of the brain renin-Ang system (RAS)

In recent years, studies have uncovered that excessive activation of the brain RAS plays an important role in the occurrence and maintenance of hypertension in various experimental and genetic hypertension animal [78,79]. The activation of RAS in the brain can improve sympathetic tone and consequently increase vascular resistance and the release of arginine vasopressin which lead to elevated BP level [80]. The main biologically active substances are Ang II and Ang III [81]. Aminopeptidase A (APA) is a membrane-bound zinc metalloprotease. It is responsible for the N-terminal cleavage of Ang II and it can convert Ang II to Ang III [82]. One study [83] demonstrated that Ang III generated by APA is one of the main effector peptides of the brain RAS, exerting tonic stimulatory control over BP in conscious hypertensive rats. Therefore, APA can be considered a candidate target for hypertension treatment.

EC33 [(*S*)-3-amino-4-mercaptobutyl sulfonic acid] [84] is a specific APA inhibitor. RB150 [4,40-dithio[bis(3-aminobutyl sulfonic acid)]] [85] is a systemically active prodrug of EC33. RB150 inhibited the biological effects of APA. Yannick et al. [86] revealed that compared with the effects of oral saline, administration of RB150 orally (100 mg/kg) in SHRs significantly inhibited brain APA activity by 31%. RB150 treatment reduced the increase in APA activity in SHRs by 58% compared with normotensive WKY rats. Additionally, oral RB150 (15–150 mg/kg) dose-dependently decreased MABP in conscious SHRs, and the ED50 was 30.5 mg/kg. The maximal decrease in MABP ($37.2 \pm 8.0$ mmHg) was observed at a dose of 150 mg/kg. The hypotensive effect peaked at 5–7 h after administration. In addition, concomitant oral administration of RB150 (100 mg/kg) with the systemic RAS blocker enalapril (1 mg/kg) significantly reduced BP in conscious SHRs within 2 h, and the maximum decrease in MABP was observed 6 h after administration. These results suggest that RB150 may be the prototype of a new class of centrally active anti-hypertensive agents, and it can be used in combination with classic systemic RAS blockers to improve BP control.

Subsequently, phase I clinical trials were conducted to evaluate the safety, pharmacokinetics and pharmacodynamic effects of RB150 in humans. The study included 56 healthy male volunteers, and the results demonstrated that RB150 was well tolerated and safe among healthy volunteers [87]. Azizi et al. conducted a pilot multicenter, double-blind, randomized, placebo-controlled, crossover pharmacodynamic study to evaluate the effects of RB150 on BP and hormones in patients with hypertension in phase II clinical trial. The study included 34 hypertensive patients who were randomly assigned to receive either RB150 and then placebo for 4 weeks each or vice versa after a 2-week run-in period. After 4 weeks, daytime ambulatory SBP had decreased by 2.7 mmHg and office SBP was decreased by 4.7 mmHg in the RB150 group versus placebo group [88]. These results suggested that RB150 can reduce daytime SBP in patients with hypertension without significant effects on systemic RAS activity. In another multicenter, open-label, phase II study, 256 overweight or obese hypertensive patients were recruited, and 54% of the participants were of Black or Hispanic. After 8 weeks of oral administration, RB150 lowered systolic automated office BP (AOBP) by 9.5 mmHg and diastolic AOBP by 4.2 mmHg. Meanwhile, the BP-lowering effects differed among the races. Systolic AOBP was decreased by 10.5 mmHg in Black participants, versus 8.9 mmHg in other participants [89]. The results of this study confirmed that RB150 has a hypotensive effect in the diverse high-risk population.

### 3.2. Vasoactive intestinal peptide (VIP) receptor

VIP is a neuropeptide that exerts positive inotropic, chronotropic and

vasodilatory effects by activating the G protein-coupled receptors VPAC1 and VPA 2 [90]. VIP is associated with several CVDs and cardiopulmonary diseases, making it a key treatment target for both systemic and pulmonary hypertension as well as HF. Vasomera (PB1046) was developed by fusing an analog of VIP with an elastin-like polypeptide. This drug exerts its effects through selectively binding VPAC2, thereby avoiding the gastrointestinal side effects associated with VPAC1 activation. In addition, PB1046 has a longer half-life than native VIP [91]. At present, two phase I randomized, double-blind, placebo-controlled studies evaluating the safety, tolerability, pharmacokinetics and pharmacodynamics of PB1046 in patients with essential hypertension have been completed (NCT 01873885, NCT 01523067), but the results have not been published.

### 3.3. Intestinal NHE3

The imbalance of sodium intake and excretion plays an important role in the pathogenesis of hypertension and its complications, such as HF and CKD [92]. NHE3 expressed on enterocytes throughout the intestinal lumen plays a dominant role in intestinal sodium absorption [93]. Therefore, inhibition of NHE3 has been considered a potential strategy for controlling hypertension and its complications. Studies indicated that oral administration of the NHE3 inhibitor tenapanor reduced the uptake of sodium in rats. In salt-fed nephrectomized rats with induced hypervolemia combined with cardiac hypertrophy and arterial stiffening, tenapanor reduced the extracellular fluid volume, left ventricular hypertrophy, albuminuria and BP. In addition, compared with enalapril alone, combined treatment with tenapanor improved cardiac diastolic dysfunction and arterial pulse wave velocity [94].

SAR218034 (SAR) is an orally nonabsorbable specific NHE3 inhibitor. Linz et al. [95] reported that in senescent lean hypertensive rats developed by feeding lean SHRs drinking water containing 0.7% NaCl, SAR (1 mg/kg per day in chow) increased fecal sodium excretion and reduced urine sodium excretion. At the same time, the drug increased feces water content and reduced SBP. In addition, the hypotensive effect of SAR can be significantly enhanced when combined with the ACE inhibitor ramipril. In hypertensive, obese and hyperinsulinemic rats (obese SHRs, not loaded with NaCl), SAR exerted a similar anti-hypertensive effect. In addition to its anti-hypertensive effect, Labonté et al. [96] demonstrated that tenapanor can protect against vascular calcification in CKD rats by decreasing serum creatinine and phosphorus levels.

### 3.4. Endothelin receptor (ETR)

Endothelin-1 (ET-1) is an endothelium-derived contractile factor released by vascular endothelial cells. To date, it is the most potent vasoconstrictor and an important factor for maintaining vascular tension [47]. ET-1 can bind with its specific receptors ETRs including ETAR and ETBR which are G-protein coupled receptors. The binding of ET-1 to ETAR can promote vasoconstriction, cell proliferation, tissue fibrosis and vascular endothelial injury, which are involved in the pathogenesis of hypertension [48]. The binding of ET-1 to ETBR can activate endothelial cells to produce NO, thereby relaxing vascular smooth muscle and inhibiting vasoconstriction and cell proliferation [49][]. Therefore, inhibition of ETAR may be a strategy for the treatment of hypertension.

At present, a variety of ETR antagonists (ETRAs) have been developed, and they can be divided into three categories according to their function: selective ETAR antagonists such as darusentan and ambrisentan; nonselective ETRAs such as bosentan and macitentan; and selective ETBR antagonists [50][]. The first anti-hypertensive ETRA used in clinical trial was bosentan. Studies have illustrated that bosentan at 0.5 or 2.0 g/day in patients with essential hypertension significantly decreased BP after 4 weeks, and its anti-hypertensive effect was comparable to that of enalapril [51]. Darusentan is a selective ETAR antagonist. A multicenter, randomized, double-blind, parallel-group, dose-response study found that darusentan dose-dependently reduced BP in patients with essential hypertension [52]. Macitentan is a novel dual ETAR/ETBR antagonist. Another study found that in Dahl salt-sensitive hypertensive and pulmonary hypertensive bleomycin-treated rats, macitentan more strongly reduced mean arterial pressure and mean pulmonary artery pressure than bosentan [53]. In addition, in a randomized, double-blind study involving 27 patients with hypertension and CKD, the ETRA sitaxentan restored a normal circadian rhythm of BP [54]. A meta-analysis of 18 studies including 4898 patients evaluating the effectiveness and safety of ETRAs in this population concluded that ETRAs significantly reduced 24-h ambulatory and sitting BP. At the office, the mean decreases in systolic and diastolic BP (DBP) were 6.12 and 3.81 mmHg, respectively, and 24-h ambulatory SBP and DPB were reduced by 7.65 and 5.92 mmHg, respectively [55]. These results illustrated that ETRAs have beneficial effects on elevated BP.

Aprocitentan is a dual ETAR/ETBR antagonist. Recently, a randomized, double-blind, parallel study was conducted in patients with essential hypertension to evaluate the anti-hypertensive effect of aprocitentan. The study included 490 patients who were administered 5, 10, 25, or 50 mg of aprocitentan, placebo or 20 mg of lisinopril as a positive control once daily for 8 weeks. The results indicated that aprocitentan at doses of 10, 25 and 50 mg lowered placebo-corrected SBP/DBP in the clinic by 7.05/4.93, 9.90/6.99 and 7.58/4.95 mmHg, respectively. In addition, placebo-corrected 24-h SBP was reduced by 3.99, 4.83 and 3.67 mmHg, respectively, by these doses, whereas 24-h DBP was reduced by 4.04, 5.89 and 4.45 mmHg, respectively. Compared with lisinopril group, the aprocitentan 25 mg group exhibited reductions of SBP and DBP of 4.84 and 3.81 mmHg, respectively. Conversely, there was no significant difference in the incidence of side effects between the aprocitentan and placebo groups [56]. At present, research on aprocitentan in combination with other drugs in the treatment of resistant hypertension is ongoing (https://www.clinicaltrials.gov; Unique identifier: NCT03541174). The results of these studies suggest that targeting ETRs may have therapeutic effects for the treatment of hypertension.

### 3.5. Drugs targeting the NO pathway

NO is a vasodilation factor that plays an important role in BP regulation [57]. NO is synthesized from L-arginine by at least three different forms of NO synthase (NOS): neuronal NO synthase, endothelial NO synthase and inducible NOS [58]. Asymmetric and symmetric dimethylarginine (ADMA and SDMA, respectively) are endogenous NOS inhibitors that can inhibit the production of NO [59]. Moreover, a recent study showed that another NO synthase inhibitor NG-nitro-L-arginine methyl ester hydrochloride (L-NAME) could induce hypertension and elicit macrophage infiltration and inflammation. The detrimental effect L-NAME-induced was reversed by metallothionein, a heavy metal-binding scavenger which indicated that NO synthase has served as a novel target [97,98]. The vascular endothelium is stimulated by shear force and other factors to induce the production and release of NO into surrounding tissues and cells, thereby reducing BP by inhibiting the vascular tone and the proliferation of smooth muscle cells. These results suggest that increasing NO levels in the body may reduce BP [60]. Therefore, NOS substrates and drugs that reduce ADMA and ADMA levels and NO donors may be useful for reducing BP.

Sphingosine-1-phosphate, the bioactive lipid mediator is a potent activator of endothelial nitric oxide synthase through G protein-coupled receptors [150]. The autocrine/paracrine activation of Sphingosine-1-phosphate receptors (SIPR) plays an important role in the regulation of BP level. Recent studies showed that S1PR1 signaling is a key pathway in BP homeostasis, genetically ablation of BP increases BP level in both physiological and pathological conditions. Moreover, an FDA-approved drug FTY702 which acts as antagonist of S1PR1 could increase BP and exacerbate hypertension in ANG II mouse model, indicating that S1PR1 may serve as a novel therapeutic target for the treatment of hypertension.

One study found that the oral administration of NOS substrates such as L-arginine or its precursor L-citrulline can lower BP in hypertensive rats. In hypertensive uremic rats, oral administration of 0.1% L-arginine lowered BP by increasing plasma NO2/NO3 levels and decreasing ET-1 levels in the aorta and kidneys. Additionally, L-arginine can alleviate kidney injury [61]. Treatment with 0.25% L-citrulline for 8 weeks significantly reduced BP in SHRs. Additionally, L-citrulline treatment dramatically altered L-arginine and ADMA levels in the kidneys of rats, thereby increasing the L-arginine/ADMA ratio. This result illustrated that NO bioavailability was restored and oxidative stress was reduced in SHR kidneys [62]. In addition, similar results were reported in clinical trials. A clinical trial found that the administration of 12 g of L-arginine daily for 4 weeks significantly reduced both SBP and DBP in patients with hypertension [63]. These results indicated that NOS substrates have protective effects on the kidneys in addition to the anti-hypertensive effects.

Drugs that reduce ADMA and SDMA levels such as resveratrol, melatonin, and *N*-acetylcysteine (NAC) can also play an important role in regulating BP. Hsu et al. [64] reported that in dexamethasone (DEX)- or 2,3,7,8-tetrachlorodibenzo-p-dioxin-exposed female rats, the administration of resveratrol reduced BP by decreasing ADMA and SDMA levels and increasing NO levels in plasma in offspring rats. In maternal rats with hypertension induced by fructose diet combined with high-salt diet feeding, oral melatonin reduced plasma AMDA and SDMA levels and protected adult offspring against programmed hypertension [65]. In offspring rats exposed prenatally to DEX and fed a high-fat diet postnatally, NAC administration reduced the levels of ADMA and restored the L-arginine/ADMA ratio in plasma, thereby preventing programmed hypertension [66].

In addition, studies indicated that NO donors such as sodium nitrate and pentaerythritol tetranitrate can also lower BP. In SHRS, plasma L-arginine and ADMA levels were decreased by treatment with 1 mmol/kg/day sodium nitrate and BP pressure was also significantly decreased [62]. Meanwhile, maternal pentaerythritol tetranitrate treatment led to a persistent BP reduction in female offspring [67]. In the future, more drugs associated with the NO pathway could be developed to reduce hypertension.

### 3.6. Vaccines

In the long history of immunotherapy, vaccines targeting the RAS for the treatment of hypertension have been reported since the 1950s [100]. Ang I and Ang II vaccines, as well as those targeting Ang receptors, can successfully lower BP in rat and mouse. An Ang I vaccine consisting of an AI analog conjugated with a tetanus toxoid carrier protein and adjuvanted with aluminum hydroxide reduced BP in Sprague-Dawley rats [101]. An Ang II-derived peptide was conjugated to the virus-like particle (VLP) Qb (AngQb). In SHR immunized with 400 mg of AngQb, the average SBP was reduced by up to 21 mmHg compared with the findings in rats administered Qb alone, and total Ang II levels (antibody-bound and free) were increased by 9-fold compared with those in the VLP controls. Then a placebo-controlled, randomized phase I trial of 12 healthy volunteers was conducted [102]. The results indicated that Ang II-specific antibody levels were elevated in all participants, giving a 100% responder rate, and AngQb was well tolerated.

The ATRQβ-001 vaccine is another peptide (ATR-001) derived from human AT1R conjugated with Qβ bacteriophage VLPs. ATRQβ-001 lowered BP by up to 35 mmHg in mice with Ang II-induced hypertension (143 ± 4 mmHg versus 178 ± 6 mmHg; $P = 0.005$) and by up to 19 mmHg in SHRs (173 ± 2 mmHg versus 192 ± 3 mmHg; $P = 0.003$), and the vaccine protected against end-organ damage caused by hypertension [103].

In addition, other studies demonstrated that in streptozotocin-induced diabetic nephropathy rats, in addition to lowering BP, ATRQβ-001 ameliorated streptozotocin-induced diabetic renal injury [104]. Some clinical trials of vaccines have also been conducted. The Ang I vaccine PMD3117 significantly increased the titers of anti-Ang I antibody in both phase I and phase II clinical trials, but it had no obvious anti-hypertensive effect [105,106]. Therefore, further studies will be required to explore whether higher titers can lead to a decrease in BP. A multicenter, double-blind, randomized, placebo-controlled clinical trial of the Ang II vaccine AngQb-Cyt006 included 72 patients with mild-to-moderate hypertension. The subjects received subcutaneous injections of either 100 or 300 μg of the conjugate (CYT006-AngQb) or placebo at weeks 0, 4 and 12. SBP and DBP were reduced by 9.0 and 4.0 mmHg, respectively, in patients in the 300 μg vaccine dose group versus the baseline levels [107]. This study confirmed that a vaccine can have a hypotensive effect in humans for the first time. In the future, the anti-hypertensive effect of vaccines must be verified in a broader population with hypertension.

### 3.7. Gastrointestinal microbiota

A large number of microbes are present in the human intestine, and these microorganisms play an important role in human health [108,109]. The abundance, diversity, and uniformity of the intestinal flora are important indicators reflecting its composition. In 2005, Eckburg et al. [110] used metagenomics to divide intestinal microorganisms into six categories: *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Actinobacteria*, *Verrucomicrobia* and *Fusobacteria*. *Bacteroidetes* and *Firmicutes* are the dominant flora, and the ratio of *Firmicutes* to *Bacteroidetes* (F/B ratio) can reflect the degree of intestinal flora disorder. Dysbiosis of the gastrointestinal microbiota is closely related to the progression of hypertension, and the metabolites of the gastrointestinal microbiota play an important role in BP regulation [111]. Mell et al. [112] were the first group to present differences between the cecal microbial components of Dahl salt-sensitive and Dahl salt-resistant rats. In the same year, Yang et al. [113] also found that compared with normotensive WKY rats, there were significant decreases in the abundance, diversity and evenness of microbial and an increase in the F/B ratio in SHR rats that were accompanied by declines in acetate- and butyrate-producing bacteria. Similar results were also found in chronic Ang II infusion rat. These results demonstrated that at the animal level, hypertension is correlated to disorders of the intestinal flora.

Recently, a study conducted in China performed comprehensive metagenomic and metabolomic analyses in a cohort of 41 healthy controls, 56 patients with pre-hypertension, and 99 individuals with primary hypertension. The results found that compared with healthy control group, microbial abundance and diversity were dramatically reduced in the pre-hypertension and hypertension groups. In addition, the counts of bacteria related to health status were reduced, whereas those of bacteria such as *Prevotella* and *Klebsiella* spp. were increased. Furthermore, by transplanting the stools of patients with hypertension into germ-free mice, BP was increased, indicating that increases in BP are transferrable through the microbiota. This study illustrated that the intestinal flora can directly regulate BP in the host [114]. The aforementioned results indicate that changes in the composition of the gut microbiota play an important role in the progression of hypertension.

It was found that after changes in the bacterial composition observed in animals and humans of hypertension, the level of bacterial metabolic end-products in the bloodstream was also changed [115]. One of the main functions of the human intestinal microbiota is to ferment indigestible dietary fiber in the large intestine. The products of this fermentation process are short-chain fatty acids (SCFAs), including acetate, propionate, and butyrate [116]. SCFAs are either absorbed by the gastrointestinal tract or excreted in feces. SCFAs mainly regulate BP by activating Olfr78 and Gpr41, both of which are G protein-coupled receptors, in vascular or renal tissues. Olfr78 is expressed in the renal juxtaglomerular apparatus, and it mediates renin secretion in response to SCFAs. Olfr78-knockout mice had lower plasma renin levels and baseline blood pressure levels than wild-type mice [117]. There are also studies revealing that an acute SCFA bolus decreases BP in anesthetized

mice, and this effect is mediated primarily via Gpr41 [118]. Therefore, Olfr78 is currently considered primarily responsible for raising BP, whereas Gpr41 is primarily responsible for lowering BP. The opposite effect of these receptors may be to regulate BP in the normal range when SCFA levels change. It has been demonstrated that the number of SCFAs produced by the intestinal microbiota is reduced in SHRs [113]. In addition, a study demonstrated that compared with Dahl salt-sensitive rats fed a normal salt diet, Dahl salt-sensitive rats fed a high-salt diet exhibited increased levels of acetate, propionate, and isobutyrate in the stool, whereas butyrate levels did not increase significantly [119].A clinical trial illustrate that high intake of fruit and vegetables, which are considered as sources of SCFAs, can reduce BP [120], and they are associated with a lower incidence of cardiovascular mortality [121]. These results were inconsistent, and thus, more studies are needed to clarify the mechanism by which SCFAs influence the development of hypertension.

A large number of studies have confirmed that regulating the intestinal microbiota may be an effective strategy for treating hypertension. Lactobacilli comprise a subdominant component of the human intestinal microbiota. Lactobacilli can enhance the release of anti-inflammatory factors. They can also reduce paracellular permeability and prevent the invasion of pathogenic bacteria [122]. A randomized, placebo-controlled study indicated that *Lactobacillus helveticus* LBK-16H-fermented milk, which contains bioactive peptides, has a BP-lowering effect in hypertensive subjects [123]. Ahren et al. [124] reported that in rats with hypertension induced by $N^G$-nitro-L-arginine methyl ester, blueberries fermented with the tannase-producing bacterium L. *plantarum* DSM 15313 can lower BP in rats and exert anti-hypertensive effect and reduce the risk of CVD. In another study, oral administration of recombinant L. *plantarum* NC8 (RLP) can express ACE inhibitory peptides and significantly reduce SBP in SHRs. Furthermore, the administration of RLP led to increased levels of NO and decreased levels of ET and Ang II in the plasma, heart and kidneys [125]. Aoyagi et al. [126] found that elderly people who drink fermented milk products containing L. *casei* strain Shirota at least three times a week had a significantly lower risk of hypertension. All the results indicate that lactobacilli can play a protective role against the development of hypertension, and probiotic intervention may be a potentially effective method for treating hypertension by restoring the intestinal microbial inhabitants. In the future, it will be necessary to further explore the mechanism of the interaction between the gastrointestinal microbiota and the host. We need to investigate the effects of different types of probiotics and explore the potential molecular mechanisms responsible for improved BP to elucidate the beneficial effect of probiotics on hypertension.

### 3.8. Leptin

Obesity lead to many adverse metabolic and cardiovascular outcomes. Among this, obesity hypertension (HTN) has gained widespread interest. Obesity is the most common cause of primary HTN and is directly proportional to increases BMI. Although the pathophysiology of obesity HTN has not yet been fully elucidated, leptin, derived from obese gene, is increasingly implicated in obesity HTN [127]. The relationship of leptin and hypertension is mutual. On the one hand, leptin increases arterial BP by activating aldosterone synthase (CYP11B2), which augments aldosterone secretion from the zona glomerulosa. On the other hand, leptin-induced cardiac contractile response was abrogated by hypertension [128]. In addition, a theory named selective leptin resistance has emerged. Selective leptin occurs in obese humans and then contribute to the sympathetic overactivity and hypertension. Study has showed that leptin administration resulted in dose-dependent suppression of weight and appetite in lean mice. Besides, ablation of leptin receptors in the subfornical organ resulted in a drop in BP level [129]. However, leptin -induced aldosterone levels failed to increase and BP reduction by adrenergic blockade was less marked in female rat which suggested that leptin failed to show the specificity of therapeutic target [127]. Whether the selective leptin antagonists have the property of antihypertensive drugs remains unclear.

### 3.9. Sodium-glucose cotransporter 2 (SGLT2)

The SGLTs are one classes of carrier protein which mediate the reabsorption of the filtered glucose. About 90% of the filtered glucose is reabsorbed in the early proximal tubule via the action of SGLT2 expressed in kidney. Inhibition of SGLT2 decreases glucose reabsorption and modestly lowers blood glucose levels. However, the ability to lower blood glucose is limited by the filtered load of glucose and the osmotic diuresis [130]. A meta-analysis of 45 placebo-controlled studies showed that SGLT2 inhibitor contributed to the mean reduction in systolic blood pressure (SBP) of −3.77 mmHg [131]. In addition, SGLT2 inhibitors seem to reduce nighttime BP compared with daytime [132]. The mechanisms of SGLT2 inhibitors lowering BP level may be via natriuresis and osmotic diuresis, but it is not clarified fully.

The first FDA-approved drug of SGLT2 inhibitor class was canagliflozin [133]. A multicenter double-blind, placebo-controlled dose-ranging study of 451 individuals randomized to escalating doses of canagliflozin (50 to 300 mg daily) added to metformin, sitagliptin, or placebo found a reduction in SBP ranging from −0.9 mmHg with 50 mg once daily to −4.9 mmHg with 300 mg once daily, compared to −1.3 mmHg with placebo and − 0.8 mmHg with sitagliptin [134]. Dapagliflozin was also a class of SGLT2 inhibitor. A randomized, placebo-controlled clinical trial showed that 10 mg dapagliflozin decreased the SBP by 4.3 mm. The effect could be explained by the synergy with β-blockers and calcium channel blockers. Furthermore, other SGLT2 inhibitors have been approved in some countries of the world, like empagliflozin, ipragliflozin, luseogliflozin and tofogliflozin.

## 4. Other factors in hypertension

### 4.1. Chemerin

Chemerin, a new relatively adipokine, can drive pathological changes in BP levels. The effect of chemerin in BP levels was mediated by the contraction of isolated arteries and the effect of established vasoconstrictors such as ET-1 [135–137]. Although the concrete molecule mechanisms which chemerin modify and participate in hypertension is not clarified fully, it plays an important role in the component of blood vessel. In endothelial cells, chemerin increases reactive oxygen species (ROS) and may lead to decreased nitric oxide (NO) production [137–139]. Chemerin is a mitogen in vascular smooth muscle cells and elevates blood pressure [140]. Moreover, chemerin promotes angiogenesis in the microvasculature [141,142]. However, the specific drugs targeting chemerin need further investigation.

### 4.2. Autophagy

Autophagy maintains endothelial cell function and the integrity of blood vessels, plays a protective role in the development of pulmonary hypertension and atherosclerosis. In the past, blood pressure overload-induced cardiac dysfunction suppressed the process of autophagy [143]. A genome-wide association study showed that a common variant in the damage-regulated autophagy modulator locus was associated with hypertension [144,145], whereas increased autophagy may account for the pulmonary arterial hypertension-induced ventricular hypertrophy and diastolic heart failure [146], which was a contradictory result of autophagy in hypertension. To date, the role of autophagy in vascular biology and blood pressure regulation remains unknown.

### 4.3. Acetylation

Acetylation and deacetylation of functional proteins are essential for hypertension. In spontaneously hypertensive rat, inhibition of histone

**Table 1**
New drug target for hypertension.

| Target | Drug | Mode of action | Status |
|---|---|---|---|
| Aminopeptidase A | Firibastat (RB150) | APA inhibitor | Phase I/II |
| Vasoactive intestinal peptide | Vasomera (PB1046) | VIP receptor agonists | Phase I |
| Na+/H+ exchanger 3 | Tenapanor | NHE3 inhibitor | |
| | SAR218034 | NHE3 inhibitor | |
| Endothelin-1 | Bosentan | Nonselective ETR antagonists | Approved |
| | Macitentan | Dual ETAR/ETBR antagonist | Approved |
| | Darusentan | Selective ETR antagonists | Approved |
| | Aprocitentan | Dual ETAR/ETBR antagonist | Approved |
| Nitric oxide | NG-nitro-L-arginine methyl ester hydrochloride | NO synthase inhibitor | Preclinical |
| | L-arginine or L-citrulline | NO synthase | Preclinical |
| | Pentaerythritol tetranitrate | NO | Preclinical |
| Sphingosine-1-phosphate | FTY702 | S1PR1 antagonist | Approved |
| Ang I and Ang II | CYT006-AngQβ | ANGII antibody | Phase II |
| | PMD3117 | ANGI antibody | Phase I/II |
| | ATRQβ-001 | ANGII type 1 receptor antibody | Preclinical |
| Sodium-glucose cotransporter 2 | Canagliflozin | SGLT2 inhibitor | Approved |
| | Dapagliflozin | SGLT2 inhibitor | Approved |

APA, Aminopeptidase A; VIP, Vasoactive intestinal peptide; NHE3, Na+/H+ exchanger 3; ETR, endothelin receptor; NO, Nitric oxide; S1PR1, Sphingosine-1-phosphate; SGLT2, Sodium-glucose cotransporter 2.

deacetylases could suppress cardiac hypertrophy and fibrosis by elevating histone 3 acetylation on promoters of MR target genes [147]. In addition, inhibition of lysine deacetylases also increased the acetylation of MR and attenuated hypertension [148]. Depletion of SIRT3 causes hyperacetylation of mitochondrial SOD2 and overproduction of oxidative stress, which results in endothelial dysfunction, vascular inflammation and hypertension in mice [149].

## 5. Perspectives

Many research fellows have realized that monotherapy in the treatment of hypertension may represent ineffectiveness due to the additional effects and adverse effects on BP control. In addition to the new targets mentioned above (Table 1), the investigation of new mechanisms and new drug targets in antihypertensive was challengeable. Thus, many companies in the market have focused on developing fixed-dose combinations (FDCs) of two or more agents [99]. In the past, ACE inhibitors can be combined with ARBs and dihydropyridine calcium-channel blockers can be combined with all other first-line antihypertensive drugs. Most late-stage antihypertensive drugs consist of dual or triple combinations, and they refer to dual or triple targets. For example, phase III trials are under way for candesartan cilexetil/nifedipine and fimasartan/amlodipine, both of which are FDCs of an ARB and a calcium channel blocker (CCB). Valsartan/amlodipine/rosuvastatin is in phase III trials while valsartan/amlodipine/atorvastatin is in phase I trials. Similar trials were also under investigation.

## 6. Conclusion

Hypertension is a common risk factor for CVD. Thus, investigating and identifying therapeutic targets for the treatment of hypertension are important. To date, some new drug classes such as AR antagonists, APA inhibitors and vaccines have been investigated in phase I/II clinical studies. In addition, drug inhibitors also have been explored in animal studies. These findings, including insights into their mechanisms of action, will facilitate the development of new anti-hypertensive drugs. However, the selectivity and efficacy of drugs targeting newly identified targets remain unknown.

It is recognized that several steps must be taken to identify new targets for hypertension therapy. First, targets must be screened and validated. Second, the appropriate and personalized daily doses of available drugs must be clarified. Finally, side effects that can lead to organ damage must be reduced.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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