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ARTICLE

Pharmacological characterization of MT-1207, a novel multitarget antihypertensive agent

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Hypertension is a serious public health problem worldwide. MT-1207, chemically named 3-(4-(4-(1H-benzotriazole-1-yl)butyl) piperazine-1-yl) benzisothiazole hydrochloride, is a new chemical entity that has entered into clinical trial as antihypertensive agent in China. In this paper we report the pharmacological profile of MT-1207 regarding its acute, subacute, and long-term effects on hypertensive animal models, and its actions on isolated organs in vitro as well as its molecular targets. Blood pressure (BP) was measured in conscious animals; amlodipine was taken as a positive control drug. We showed that both single dose of MT-1207 (1.25−20 mg/kg, ig) in spontaneously hypertensive rats (SHR) and MT-1207 (0.25−6 mg/kg, ig) in two-kidney one-clip (2K1C) dogs dose-dependently decreased BP. MT-1207 quickly decreased BP within 5 min after administration; the hypotensive effect lasted for 8 and 12 h, respectively, in SHR and 2K1C dogs without reflex increase in heart rate. Multiple doses of MT-1207 (5 $mg \cdot kg^{-1} \cdot d^{-1}$ in SHR; 2 $mg \cdot kg^{-1} \cdot d^{-1}$ in 2K1C dogs, for 7 days) significantly decreased BP, slightly reduced heart rate, and both of them recovered after withdrawal. Long-term administration of MT-1207 (10 $mg \cdot kg^{-1} \cdot d^{-1}$ for 4 months or more time) produced a stable BP reduction, improved baroreflex sensitivity, reduced renal and cardiovascular damage in SHR, and delayed stroke occurrence and death in stroke-prone SHR. In isolated rat aortic rings precontracted by adrenaline, KCl, noradrenaline or 5-hydroxytryptamine (5-HT), MT-1207 (10^{-9}–10^{-4} M) caused concentration-dependent relaxation. In a panel of enzyme activity or radioligand binding assays of 87 molecular targets, MT-1207 potently inhibited adrenergic α_{1A}, α_{1B}, α_{1D}, and 5-HT_{2A} receptors with $K_i < 1$ nM. The antagonism of MT-1207 against these receptors was confirmed in isolated rabbit arteries. We conclude that MT-1207 is a novel and promising single-molecule multitarget agent for hypertension treatment to reduce hypertensive organ damage and stroke mortality.

Keywords: blood pressure; antihypertensive agent; MT-1207; amlodipine; multitarget; α_1 receptor; 5-HT_{2A} receptor; organ protection; spontaneously hypertensive rats; two-kidney one-clip dogs

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INTRODUCTION

Hypertension is one of the most serious public health problems worldwide. The global prevalence of hypertension is ~1.13 billion [1], with a prevalence of ~245 million in China [2]. In addition to elevated blood pressure (BP), hypertension induces structural and/or functional damage in major organs (i.e., the heart, brain, retina, kidney, and vasculature) [1–3]. Therefore, hypertension is a major risk factor for cardiovascular and renal events, including stroke, myocardial infarction, sudden death, heart failure, peripheral artery disease, and end-stage renal disease. Importantly, hypertension is the leading global contributor to premature death. High systolic BP accounts for most of the mortality and disability burden (70%), and the largest number of systolic BP-related deaths are due to stroke and myocardial infarction. Because of the serious harm caused by hypertension, the goal of antihypertensive treatment is to effectively reduce not only BP but also organ damage.

There are more than 10 classes of antihypertensive drugs with more than 50 kinds of drugs [4]. However, only 35% of treated hypertensive patients globally are controlled to a BP of less than 140/90 mmHg [1], with a BP control rate of 16.9% in China [2], indicating very low control of BP worldwide. Furthermore, despite advances in diagnosis and treatment over the past 30 years, the disability-adjusted life years attributable to hypertension have increased by 40% since 1990 [5]. Thus, it is necessary to adopt new therapeutic strategies and develop new antihypertensive drugs.

BP is regulated by multiple systems with multiple mechanisms. Single-target antihypertensive drugs can only regulate one aspect, which makes the control of BP unsatisfactory. Increasing the dosage produces little additional BP lowering but may increase the risk of adverse effects. Evidence-based medicine demonstrates that the majority of patients require combination therapy to control BP [1]. Initial combination therapy is invariably more effective at lowering BP than monotherapy; indeed, even low-

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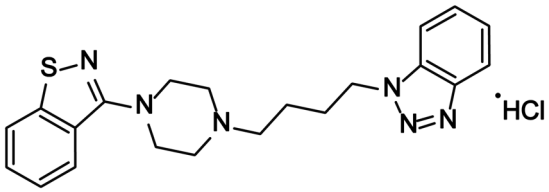

Fig. 1 Chemical structure of MT-1207.

dose combination therapy is usually more effective than maximal dose monotherapy. Furthermore, the combination of drugs targeting multiple mechanisms reduces the heterogeneity of the BP response to initial treatment and provides a steeper dose response than that observed with escalating doses of monotherapy. Thus, the current drug treatment strategy for hypertension emphasizes combination therapy targeting multiple mechanisms. The development of multitarget drugs for antihypertensive treatment is desirable.

Multitarget drugs can be divided into three categories. The first category, "multidrug multitarget", refers to the use of two or more single-target drugs at the same time and the use of synergy to achieve the goal of BP control. However, due to complex drug delivery schemes, increasing the numbers of single-target drugs doubles the risk for patients because of multiple drug interactions, increased burden in the liver and kidney, or poor compliance. To improve adherence to treatment for good compliance, a combination of two or three single-target drugs in a single pill (i.e., fixed-dose combination), the second type of multitarget drug, is preferably recommended for hypertension treatment [1]. This single-pill combination therapy is simple and pragmatic for most hypertensive patients. Many two-drug single-pill combinations have been developed and largely comprise an ACEI (or ARB) and a CCB (or diuretic). We have developed a new single-pill combination comprising a CCB (nitrendipine) and a β-blocker (atenolol) [6, 7], which has been widely used in China since 2009 [2]. According to our experience, the key for research and development of single-pill combinations is the selection of an optimal ratio of the combined drugs for preferable synergy, which can be determined by using the probability sum test (*q* test) [6–9]. Pharmacokinetic synchronism must also be considered. Using an optimal ratio of the combined drugs may produce maximal synergism; indeed, even a combination of single drugs at an ineffective dose can achieve effective BP reduction [6, 7], and it always protects against hypertensive organ damage [7, 8]. The reduced dose in a single-pill combination results in a reduction in risk (adverse effects) along with a low cost. However, a single-pill combination is often not feasible when there are more than three active ingredients. Additionally, a single-pill combination is normally developed with approved drugs. Otherwise, it is too expensive for the development of such new drug, since each new component in the combination must be approved separately by the drug administration. The third type of multitarget drug is a "single-molecule multitarget", that is, one drug that can act on multiple targets at the same time. This kind of multitarget drug is characterized by an effect on multiple targets and a synergistic effect on each target, which ultimately maximizes the drug efficacy and minimizes the side effects. Research and development of "single-molecule multitarget" drugs is the trend of modern multitarget drug research. At present, almost no "single-molecule multitarget" drugs have been approved for hypertension treatment.

MT-1207 (also known as X7 in preclinical research), with the chemical name 3-(4-(4-(1H-benzotriazole-1-yl)butyl)piperazine-1-yl) benzisothiazole hydrochloride (Fig. 1), is a new chemical entity that entered into clinical trials in China as an antihypertensive agent in 2018. Here, we present the pharmacological profile of this novel multitarget antihypertensive agent, focusing on its acute, subacute and long-term effects on hypertensive animal models, as well as its actions on isolated organs and molecular targets in vitro.

MATERIALS AND METHODS

Animals and experimental hypertensive model preparation

Animal experiments were approved by the Institutional Animal Care and Use Committee of the Second Military Medical University. Animals were randomly divided into different groups and selected for all experiments by laboratory technicians. Adult spontaneously hypertensive rats (SHR) [10] and stroke-prone spontaneously hypertensive rats (SHR-SP) [11] were provided by the animal center of the Second Military Medical University. Sprague-Dawley rats, beagle dogs, rabbits, and guinea pigs were purchased from Sino-British SIPPR/BK Lab Animal Ltd. (Shanghai, China) and Shanghai Jiagan Biotechnology Ltd. (Shanghai, China). Male SHR (250 ± 20 g) were used in acute and subacute experiments. Both male SHR (350 ± 20 g) and female SHR (200 ± 20 g) were used in long-term treatment studies. Male SHR-SP (350 ± 20 g) were used in long-term treatment studies. In the organ chamber study, male Sprague-Dawley rats (200 ± 20 g) and both male and female rabbits (2–3 kg) were used for the preparation of isolated blood vessels. Male guinea pigs (240–250 g) were used for preparation of isolated hearts. Both males and females beagle dogs (8–10 kg) were used to establish renovascular hypertensive models. All animals were housed under controlled conditions (temperature 23–25 °C and lighting 8:00–20:00) and received standard animal chow and tap water *ad libitum*.

Two-kidney one-clip (2K1C) dogs were prepared as a renovascular hypertensive model as we described previously [12]. Briefly, the dogs were anesthetized by intravenous injection of sodium pentobarbital (30 mg/kg). After a median incision of the upper abdomen, the right renal artery was exposed and constricted to reduce the blood flow to ~30%–40% of the baseline value with nylon threads. At 45 days after the operation, BP was measured by a noninvasive ultrasonic Doppler BP system (DS-100, Hefei Golden Brains Optical Instrument Co., Ltd, Hefei, China), and 2K1C dogs with systolic BP ≥ 160 mmHg were used for drug administration experiments.

Drugs

MT-1207 was synthetized (batch number 2013103001, HPLC purity: 99.0%) with mass spectrum features as follows: ^{1}H-NMR (600 MHz, *d*-DMSO) δ ppm 1.72–1.77 (m, 2H), 2.00–2.05 (m, 2H), 3.21 (t, $J = 7.8$ Hz, 2H), 3.29–3.34 (m, 4H), 3.57 (s, 2H), 4.07 (s, 2H), 4.80 (t, $J = 7.2$ Hz, 2H), 7.45–7.51 (m, 2H), 7.60–7.63 (m, 2H), 7.93 (d, $J = 8.4$ Hz, 1H), 8.06–8.13 (m, 3H); ^{13}C-NMR (150 MHz, *d*-DMSO) δ ppm 162.30, 152.21, 145.27, 132.88, 128.24, 127.34, 127.06, 124.73, 124.11, 121.32, 119.26, 110.76, 54.98, 50.65, 46.96, 46.46, 26.60, 20.60; HRMS (ESI): m/z calcd for $C_{21}H_{25}SN_6$ ($M + H^+$) 393.1856, found 393.1991.

Amlodipine besylate was purchased from Pfizer Pharmaceuticals Co., Ltd. (batch number L02430, Dalian, China). Other agents purchased were ketamine hydrochloride injection (batch number 130303, Fujian Gutian Pharmaceutical Co., Ltd., Ningde, China), diazepam injection (batch number 1403031, Henan Anyang Yikang Pharmaceutical Company, Anyang, China), sodium pentobarbital (batch number WS20130112, Shanghai Boguang Biotechnology Co., Ltd., Shanghai, China), adrenaline hydrochloride (batch number 10150901, Shanghai Harvest Pharmaceutical Co., Ltd., Shanghai, China), noradrenaline bitartrate injection (batch number 08151102, Shanghai Harvest Pharmaceutical Co., Ltd.), KCl (batch number F20050713, Sinopharm Chemical Reagent Co., Ltd., Shanghai, China), 5-hydroxytryptamine (5-HT, SP batch number LUC0O74, Beijing J&K Scientific Ltd., Beijing, China) and (R)-phenylephrine hydrochloride (batch number GJ01-TESP, TCI Industrial Development Co., Ltd., Shanghai, China).

Single intragastric administration of drugs and BP measurement in conscious unrestrained SHR

As we described previously [13], SHR were anesthetized with an intraperitoneal injection of ketamine (50 mg/kg) and diazepam

(5 mg/kg). A polyethylene catheter was inserted into the lower abdominal aorta via the left femoral artery for BP measurement, and another catheter was placed into the stomach via a midabdominal incision for drug administration. The catheters were exteriorized through the interscapular skin. After a 24 h recovery period, the animals were individually housed for BP recording in cylindrical cages containing food and water. The aortic catheter was connected to a BP transducer via a rotating swivel that allowed the animals to move freely in the cage. After ~2 h for stabilization, the BP signal was recorded in conscious rats for 1 h to serve as the basal value. Thereafter, a single dose of MT-1207 (1.25, 2.5, 5, 10, 15, or 20 mg/kg), vehicle, or amlodipine besylate (5 mg/kg) was given via the intragastric catheter to SHR in the corresponding dose group ($n = 10$ per group). BP and heart rate were continuously recorded for 24 h and calculated at the corresponding time to serve as the data after drug administration.

Single administration of drugs by gavage and BP measurement in conscious 2K1C renovascular hypertensive dogs

The 2K1C dogs with systolic BP ≥ 160 mmHg were anesthetized by intravenous injection of sodium pentobarbital (30 mg/kg), and a polyethylene catheter was inserted into the left femoral artery for BP measurement. After a 24 h recovery period, the catheters of the dogs were connected to a BP transducer and PowerLab 8/30 system (ML870/P, ADInstruments Pty Ltd, Bella Vista, Australia) to record BP. After stabilization for 30 min, BP and heart rate were recorded in conscious dogs for 10 min to serve as the basal value. Thereafter, a single dose of MT-1207 (0.25, 0.5, 1, 1.5, 2, 4, or 6 mg/kg), vehicle, or amlodipine besylate (1.5 mg/kg) was given by gavage in the corresponding dose group ($n = 6$ per group). BP and heart rate were continuously recorded for 120 min and then recorded for 5 min every 2 h for 24 h after drug administration.

Multiple administration of drugs by gavage and measurement of BP and electrocardiogram in SHR and 2K1C renovascular hypertensive dogs

Studies were performed in SHR (MT-1207 5 $mg \cdot kg^{-1} \cdot d^{-1}$, $n = 12$) and 2K1C dogs (MT-1207 2 $mg \cdot kg^{-1} \cdot d^{-1}$, $n = 6$). BP was measured by a noninvasive BP system (rats: ALC-NIBP, Alcott Biotech Co., Ltd., Shanghai, China; dogs: DS-100, Hefei Golden Brains Optical Instrument Co., Ltd., Hefei, China) every day for 7 days. On the 7th day, electrocardiogram (ECG) indexes were measured in anesthetized rats and conscious dogs by a PowerLab 8/30 system (ML870/P, ADInstruments Pty Ltd., Bella Vista, Australia) as the basic values. Then, the rats and dogs were administered MT-1207 by gavage once a day for 7 consecutive days, and BP was measured 60 min after each administration. ECG indexes were measured again on the 7th day after administration as the postadministration value. After stopping administration, BP was measured every day for 7 days, and ECG indexes were measured again on the 7th day.

Long-term treatment of drugs mixed in rat chow and measurement of BP, baroreflex sensitivity (BRS), and organ morphology in SHR

Studies were performed in three groups of SHR: vehicle control group, 5 $mg \cdot kg^{-1} \cdot d^{-1}$ amlodipine group and 10 $mg \cdot kg^{-1} \cdot d^{-1}$ MT-1207 group ($n = 20$ per group). Amlodipine and MT-1207 were mixed in the rat chow. The consumption of rat chow-containing drugs was measured. The content of drugs in the rat chow was calculated according to the chow consumption, and the ingested doses of amlodipine and MT-1207 were 5 and 10 mg/kg per day, respectively. The vehicle control group received the same diet without the drugs. Before administration, the baseline BP of SHR was measured by noninvasive pressure measurement of the tail artery (ALC-NIBP, Alcott Biotech Co., Ltd., Shanghai, China) every other day for 3 consecutive data points. The average value of three BP measurements was taken as the baseline value. After drug administration, the tail artery pressure was measured every week for the first 3 weeks and then measured every month during the treatment.

After 4 months of treatment, BRS was determined in conscious unrestrained SHR. Briefly, rats were anesthetized with a single intraperitoneal injection of ketamine (50 mg/kg) and diazepam (5 mg/kg). A polyethylene catheter was inserted into the lower abdominal aorta via the left femoral artery for BP measurement, and another catheter was inserted into the femoral vein for drug administration. The catheters were exteriorized through the interscapular skin. After a 24 h recovery period, the animals were housed individually for BP recording in cylindrical cages containing food and water. The aortic catheter was connected to a BP transducer via a rotating swivel that allowed the animals to move freely in the cage. After ~2 h for stabilization, the BP signal was recorded in conscious rats for 1 h to serve as the basal value. Thereafter, BRS was determined as we described previously [7]. While recording BP and heart period, ~5 μg/kg phenylephrine hydrochloride was injected intravenously through a venous catheter to induce an elevation of systolic BP between 20 and 50 mmHg. After BP recovered to the basal level, this process was repeated twice. BRS was calculated using our previously described method [7].

Morphological examinations were carried out after BRS measurement. The animals were weighed and anesthetized by a single intraperitoneal injection of sodium pentobarbital (60 mg/kg). The bilateral kidneys were removed and weighed. Ratios of right kidney weight to body weight (RKW/BW) and left kidney weight to body weight (LKW/BW) were calculated. The brain, heart, right kidney, and thoracic aorta of number 1, 3, and 5 animals in each group were used to prepare pathological sections, and hematoxylin-eosin staining was performed for pathological observation and scoring.

For the semiquantitative evaluation of glomerular damage, the glomerulosclerosis score was defined as we previously described [7]. On the light microscopic specimens, ~50 glomeruli from the outer cortex and the same number of glomeruli from the inner cortex of each kidney were graded according to the degree of sclerosis: 0, no mesangial expansion; (1) mild mesangial expansion (<30% of a glomerular area); (2) moderate mesangial expansion (30%–60% of a glomerular area); (3) marked mesangial expansion (>60% of a glomerular area); and (4) global sclerosis. This was performed by one observer in a blind fashion using coded slides. A weighted composite sclerosis score was then calculated for each kidney according to the following formula: glomerulosclerosis score = [1 × (number of grade 1 glomeruli) +2 × (number of grade 2 glomeruli) + 3 × (number of grade 3 glomeruli) + 4 × (number of grade 4 glomeruli)]/(number of glomeruli observed).

Long-term treatment of drugs mixed in rat chow and measurement of BP and survival rate in SHR-SP

Studies were performed in three groups of SHR-SP: vehicle control group, 5 $mg \cdot kg^{-1} \cdot d^{-1}$ amlodipine group, and 10 $mg \cdot kg^{-1} \cdot d^{-1}$ MT-1207 group ($n = 10$ per group). Amlodipine and MT-1207 were mixed in rat chow as mentioned above. The ingested doses of amlodipine and MT-1207 were 5 and 10 mg/kg per day, respectively. The vehicle control group received the same diet without the drugs. During the experiment, 1% sodium chloride was added to the drinking water to accelerate the occurrence of stroke. Before drug administration, the basal BP of SHR-SP was measured by noninvasive pressure measurement of the tail artery every other day for 3 consecutive data points. The average value of three BP measurements was taken as the baseline value. After drug administration, the tail artery pressure was measured every month for 4 months. At the same time, animal death in each group was observed and recorded every day.

Organ chamber studies of drug effects on vascular function

The vascular relaxation effect of MT-1207 was determined in precontracted aortae induced by various vasoconstrictors using our previously described method [14]. Sprague-Dawley rats were anesthetized by intraperitoneal injection of sodium pentobarbital (40 mg/kg). After opening the thoracic cavity, the descending thoracic aorta was immediately removed and placed in cold Krebs–Henseleit solution of the following composition (mM): NaCl 118.4, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25.0, glucose 11.1 and $CaNa_2$-EDTA 0.026 and aerated with 95% O_2 and 5% CO_2. The rat aorta was cut into 3-mm wide rings, and aortic rings were prepared by removing the endothelium and adventitial fat. The aortic rings were suspended in conventional organ baths filled with 20 mL Krebs–Henseleit solution, maintained at 37 °C and continuously aerated with 95% O_2 and 5% CO_2. Changes in isometric tension were recorded using an ALC-M tissue organ bath system (Alcott Biotech Co., Ltd., Shanghai, China). Each aortic ring was allowed to equilibrate for 60 min at a resting tension of 1.0 g. After 60 min of equilibration, corresponding vasoconstrictor drugs (adrenaline 10^{-5} mol/L, KCl 60 mmol/L, noradrenaline bitartrate 10^{-5} mol/L, or 5-HT 10^{-5} mol/L) were added to the bath solution to precontract the aortic rings. After several washes, the aortic rings were re-equilibrated for 60 min before the formal experiment. To determine the relaxant effect of MT-1207, the aortic rings were precontracted again by the corresponding vasoconstrictors, and once sustained tension was obtained, MT-1207 (10^{-9}–10^{-4} M) was added cumulatively to induce a concentration-dependent response.

In additional sets of experiments, the antagonistic effects of MT-1207 on α_1- and 5-HT receptor agonists were determined. As soon as the rabbits were anesthetized by intravenous injection of sodium pentobarbital (30 mg/kg), the thoracic cavity was opened, and the descending thoracic aorta was immediately removed and placed in cold Krebs–Henseleit solution aerated with 95% O_2 and 5% CO_2. The rabbit aorta was cut into 4-mm wide rings with the endothelium and adventitial fat removed. The aortic rings were suspended in conventional organ baths filled with 20 mL Krebs–Henseleit solution, maintained at 37 °C and continuously aerated with 95% O_2 and 5% CO_2. Changes in isometric tension were recorded. Each aortic ring was allowed to equilibrate for 120 min at a resting tension of 1.5 g. After equilibration, corresponding vasoconstrictor drugs (phenylephrine 10^{-6}–(6×10^{-3}) mol/L or 5-HT 10^{-8}–(3×10^{-4}) mol/L) were added cumulatively to the bath solution to induce concentration-dependent aortic contraction. After washing with Krebs–Henseleit solution repeatedly, the aortic rings were re-equilibrated for 60 min before the formal experiment. MT-1207 (3×10^{-8} mol/L for phenylephrine or 10^{-7} mol/L for 5-HT) was added and incubated for 20 min. Then, the corresponding vasoconstrictors were added cumulatively again to induce aortic contraction.

Organ chamber studies of drug effects on isolated heart function

After anesthesia of the guinea pigs, each chest was opened, and the heart (including part of the superior vena cava and ascending aorta) was quickly removed and immersed in cold Krebs–Henseleit solution. After removal of adipose tissue and blood clots, a fixed metal tube was inserted into the aorta. The isolated heart was placed on a Langendorf device, and aortic retrograde perfusion was performed at a constant pressure of 80 mmHg. The perfusate (Krebs–Henseleit solution) was saturated with 95% O_2 and 5% CO_2, the temperature was maintained at 36.5 ± 1 °C, the pH was adjusted to 7.35 ± 0.05, and the volume flow of the coronary artery was adjusted to 10–12 mL/min to keep the heart in a normal beating state. After a 15 min stable period, Labchart software was used to record the basic heart rate values and the data 30 min after administration of MT-1207.

Binding inhibition studies of drug effects on molecular targets

The binding inhibition activities of MT-1207 were evaluated by Shanghai Medicilon, Inc. (Shanghai, China). Briefly, in the preliminary screening experiment, the effects of 1 μM MT-1207 on enzyme activity or radioligand binding of 87 molecular targets were detected, and the experiment was repeated at least twice. The results were expressed as the specific binding inhibition percentage of the radioligand or the inhibition percentage of enzyme activity. Molecular targets with inhibition percentages ≥50% by MT-1207 entered into the secondary screening. In the secondary screening experiment, the activity of MT-1207 was measured using radioligand binding assays, and five concentrations (0.1 nM, 1 nM, 10 nM, 0.1 μM, 1 μM) of MT-1207 were used to measure the dose-effect relationship with the target molecule. The experiment was repeated at least twice. The results were expressed as inhibition percentages, and the IC_{50} (concentration at 50% inhibition), K_i (inhibition constant), and n_H (Hill coefficient) were calculated.

Binding mode study of MT-1207 with the 5-HT_{2A} receptor

The crystal structure of the 5-HT_{2A} receptor was obtained from a protein database bank (PDB ID: 6A93) [15] and prepared for docking using the protein preparation tool in Discovery Studio 3.0. During this process, the ligands and waters were removed, and hydrogens were added to the structure. Staged minimization was performed with default settings. The docking studies were carried out using GOLD 5.0 [16]. The binding site was defined as whole residues within a 10 Å radius subset encompassing the ligand. Conformations were generated by a genetic algorithm and scored using GoldScore as the fitness function. The best conformation was chosen to analyze the ligand–protein interaction. The image representing the best position was prepared using PyMol.

Statistical analysis

Data were expressed as the mean ± SEM. Comparisons between values obtained in the same group before and after drug administration were made using paired Student's *t*-tests. Comparisons between groups were made using unpaired Student's *t*-test. Survival analysis between the treatment group and vehicle control group was performed using the log-rank test. "*n*" represents the number of specimens or animals tested in each group. $P < 0.05$ was considered statistically significant.

RESULTS

Acute effect of MT-1207 single administration on BP and heart rate in SHR

As shown in Fig. 2a and Supplementary Information Fig. S1, 15 min after intragastric administration, the BP of all MT-1207 dose groups decreased compared with that before administration. The maximal decrease in systolic BP at 2 h post administration was 17 mmHg in the 1.25 mg/kg group and 21 mmHg in the 2.5 mg/kg group (Fig. 2a, b). In the 10 mg/kg group, the maximal decrease in systolic BP was 41 mmHg. There was an obvious dose-effect relationship with the 2.5, 5, and 10 mg/kg concentrations. However, the antihypertensive effect did not increase with increasing doses over 10 mg/kg (Fig. 2b and Supplementary Information Fig. S2). Taking a systolic BP reduction of 20 mmHg as the criterion for effective hypotension, we set MT-1207 2.5 mg/kg as the low-dose group, 5 mg/kg as the medium-dose group and 10 mg/kg as the high-dose group.

In the experiment studying the BP reduction onset time of MT-1207 (Fig. 2c), within 2 h post administration, the systolic BP of the MT-1207 group (5 mg/kg) decreased by 12 mmHg at 2.5 min and 20 mmHg at 5 min after intragastic administration. The maximal hypotensive effect occurred at 30 min after administration. Compared with amlodipine, a CCB commonly used in

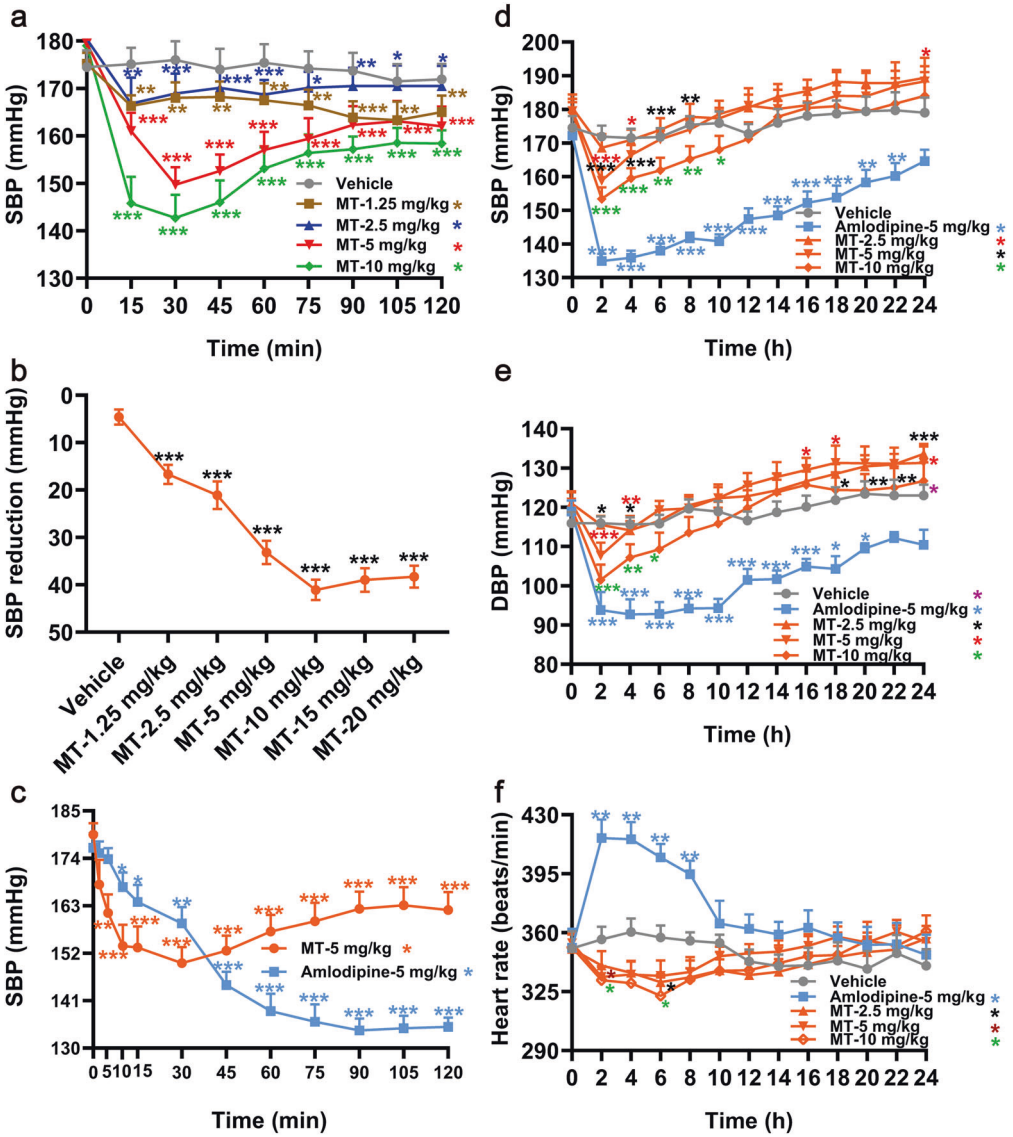

Fig. 2 Acute effect of MT-1207 single administration on blood pressure and heart rate in spontaneously hypertensive rats (SHR). a Dose effect of MT-1207 on systolic blood pressure (SBP) in SHR after a single administration. **b** Maximum reduction in SBP corresponding to **a**. **c** Onset time of hypotensive action after a single administration of MT-1207 or amlodipine at 5 mg/kg in SHR. Effects of different doses of MT-1207 on SBP (**d**), diastolic blood pressure (DBP) (**e**), and heart rate (**f**) in SHR after a single administration. MT, MT-1207. Data are shown as the mean ± SEM. $n = 10$ per group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus h 0 (before drug administration).

antihypertensive treatment, MT-1207 had a more rapid onset of its hypotensive effect. Amlodipine (5 mg/kg) decreased systolic BP by 9 mmHg at 10 min after administration and by over 20 mmHg at 45 min after administration, and its maximal hypotensive effect was achieved 90 min after administration.

We further analyzed the 24-h BP and heart rate measurement data in conscious rats after drug administration (Fig. 2d–f, Supplementary Information Fig. S3a). The BP of MT-1207 in the three dose groups (2.5, 5, and 10 mg/kg) decreased significantly within 8–10 h after administration, and the decrease in BP was dose dependent; that is, the larger the dose was, the more obvious the decrease in BP. The maximal decrease in BP occurred within 2 h after administration. The time to maintain systolic BP reduction was 8–10 h. After 12 h, the BP gradually increased approaching the preadministration level. During BP reduction, MT-1207 did not increase heart rate but slightly decreased heart rate. However, in the amlodipine group, BP reduction was accompanied by a significant increase in heart rate (Fig. 2f).

MT-1207 had a significant dose-dependent relationship with the decrease in systolic and diastolic BP. The maximal BP reduction also occurred within 2 h after administration. The time maintaining the decrease of systolic BP was more than 12 h. During BP reduction, MT-1207 did not induce a reflex increase in heart rate but decreased heart rate. However, amlodipine induced a reflex increase in heart rate with a reduction in BP (Fig. 2f).

Acute effect of MT-1207 single administration on BP and heart rate in 2K1C renovascular hypertensive dogs

As shown in Fig. 3a, b, MT-1207 dose-dependently lowered systolic BP in 2K1C dogs, with a maximal reduction of 55 mmHg after drug administration by gavage. The antihypertensive effect did not increase with dosages higher than 4 mg/kg. According to the results in Fig. 3a, b, we set 1 mg/kg MT-1207 as the low-dose group, 2 mg/kg as the medium-dose group and 4 mg/kg as the high-dose group.

In the experiment studying the BP reduction onset time of MT-1207, as shown in Fig. 3c, within 2 h post administration, the

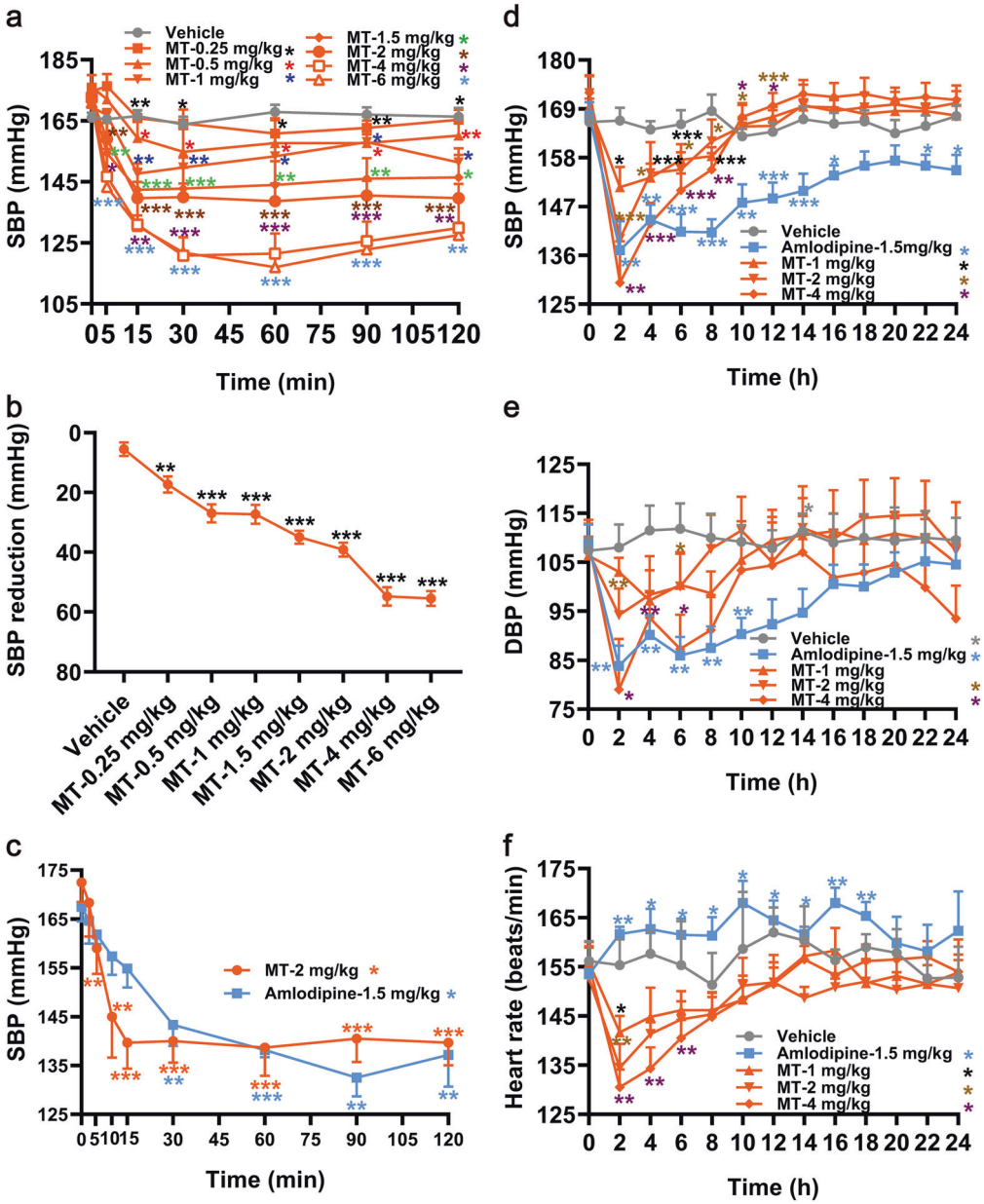

Fig. 3 Acute effect of MT-1207 single administration on blood pressure and heart rate in two-kidney one-clip (2K1C) renovascular hypertensive dogs. a Dose effect of MT-1207 on systolic blood pressure (SBP) in 2K1C dogs after a single administration. **b** Maximum reduction in SBP corresponding to **a**. **c** Onset time of hypotensive action after a single administration of MT-1207 at 2 mg/kg or amlodipine at 1.5 mg/kg in 2K1C dogs. Effects of different doses of MT-1207 on SBP (**d**), diastolic blood pressure (DBP) (**e**), and heart rate (**f**) in 2K1C dogs after a single administration. MT, MT-1207. Data are shown as the mean ± SEM. $n = 6$ per group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus h 0 (before drug administration).

systolic BP of the MT-1207 group (2 mg/kg) decreased by 14 mmHg at 5 min and by more than 20 mmHg at 10 min. Compared with the MT-1207 group, the amlodipine group (1.5 mg/kg) had a slower hypotensive effect. The BP decreased by 20 mmHg at 30 min after administration.

We further analyzed the 24-h BP and heart rate measurement data post administration (Fig. 3d–f, Supplementary Information Fig. S3b). MT-1207 had a significant dose-effect relationship with the decrease in systolic and diastolic BP. The maximal BP reduction also occurred within 2 h after administration. The time required to maintain the decrease in systolic BP was more than 12 h. During BP reduction, MT-1207 did not induce a reflex increase in heart rate but decreased heart rate. However, amlodipine induced a reflex increase in heart rate with a reduction in BP (Fig. 3f).

Subacute effect of multiple administrations of MT-1207 for 1 week on BP, heart rate, and ECG in SHR

Before administration, the BP of SHR was measured for 1 week. As shown in Table 1, the average systolic BP was 175 ± 0.99 mmHg. After administration of MT-1207 (5 mg/kg) by gavage once daily for 1 week, the average systolic BP dropped to 140 ± 2.43 mmHg. After drug withdrawal, systolic BP returned to the level measured before administration.

One week before administration, the average heart rate of SHR was 346 ± 21.88 beats per minute (bpm). During 1 week of administration, the heart rate did not increase with the decrease in BP but slightly slowed down, with an average of 326 ± 16.39 bpm. After drug withdrawal, the heart rate was 329 ± 19.65 bpm. There was no significant difference from the baseline value (Table 1).

Table 1. Effect of multiple administration of MT-1207 for 1 week on blood pressure, heart rate, and electrocardiogram in SHR and 2K1C renovascular hypertensive dogs.

	SHR			2K1C		
	Baseline	MT-1207 (5 mg/kg)	Withdrawal	Baseline	MT-1207 (2 mg/kg)	Withdrawal
SBP (mmHg)	175 ± 0.99	140 ± 2.43***	176 ± 1.59	173 ± 1.45	139 ± 1.38***	174 ± 1.58
HR (bpm)	346 ± 21.88	326 ± 16.39	329 ± 19.65	150 ± 3.66	138 ± 4.75*	147 ± 3.19
QT (s)	0.07 ± 0.004	0.08 ± 0.003	0.08 ± 0.003	0.19 ± 0.004	0.20 ± 0.007	0.19 ± 0.005
QRS (s)	0.02 ± 0.001	0.02 ± 0.001	0.02 ± 0.001	0.03 ± 0.001	0.04 ± 0.001	0.03 ± 0.001
T wave (s)	0.04 ± 0.003	0.04 ± 0.004	0.04 ± 0.003	0.03 ± 0.002	0.05 ± 0.010	0.04 ± 0.005

Data are shown as mean ± SEM. *$P < 0.05$, ***$P < 0.001$ vs baseline. $n = 12$ in SHR and $n = 6$ in 2K1C renovascular hypertensive dogs. MT-1207 was administered by gavage once a day for 7 consecutive days.
SBP systolic blood pressure, *HR* heart rate, *QT* QT interval, *QRS* QRS duration, *SHR* spontaneously hypertensive rats, *2K1C* two-kidney one-clip dogs.

According to the analysis of QT interval duration, QRS duration and T wave duration, multiple administrations of MT-1207 for 1 week had no significant effect on the ECG of SHR (Table 1).

Subacute effect of MT-1207 multiple administration for 1 week on BP, heart rate, and ECG in 2K1C renovascular hypertensive dogs
The systolic BP at baseline before administration was 173 ± 1.45 mmHg (Table 1). Then, MT-1207 (2 mg/kg) was administered by gavage once a day for 7 consecutive days. The average systolic BP during the treatment was 139 ± 1.38 mmHg. After drug withdrawal, the average systolic BP was 174 ± 1.58 mmHg, which recovered to the level measured before administration.

The heart rate of 2K1C dogs changed from 150 ± 3.66 bpm before administration to 138 ± 4.75 bpm ($P < 0.05$ vs baseline) after MT-1207 treatment (Table 1). The heart rate slowed slightly compared with the baseline heart rate. After drug withdrawal, the heart rate returned to the level observed before administration.

On the 7th day after continuous administration, the QT interval duration and QRS duration were both prolonged by 0.01 s, and the T wave duration was prolonged by 0.02 s. However, these indexes were neither significantly different compared with the baseline values nor outside of their physiological fluctuation ranges (Table 1). They all recovered to the baseline level after drug withdrawal.

Long-term effect of MT-1207 treatment for 4 months on BP, BRS, and organ protection in SHR
Systolic BP decreased by more than 20 mmHg 1 week after MT-1207 treatment ($10\,mg \cdot kg^{-1} \cdot d^{-1}$) and remained stable during 4 months of treatment, similar to amlodipine treatment ($5\,mg \cdot kg^{-1} \cdot d^{-1}$) (Fig. 4a), which was significantly different from that of the vehicle control group. At the end of treatment, the BRS of SHR in the vehicle control group was 0.27 ± 0.01 ms/mmHg, while that in the amlodipine and MT-1207 treatment groups was 0.55 ± 0.03 ms/mmHg and 0.53 ± 0.03 ms/mmHg, respectively. Compared with the vehicle control group, the BRS was significantly improved in the two treatment groups (Fig. 4b).

Further, pathological examination of the long-term treated SHR was performed (Fig. 4c, d). MT-1207, similar to amlodipine, did not reduce the kidney weights of SHR but showed a trend toward increasing kidney weights. Both MT-1207 and amlodipine treatments significantly decreased glomerular sclerosis scores in SHR. The histological changes after long-term treatment also supported a renoprotective effect for both MT-1207 and amlodipine (Fig. 4e). Additionally, the pathological changes in the heart, brain and aorta in the amlodipine and MT-1207 groups were improved compared with those in the vehicle group (data not shown).

Effect of long-term treatment with MT-1207 on BP and survival rate in SHR-SP
Six-month-old SHR-SP were randomly divided into three groups with ten rats in each group. They were treated with vehicle control, amlodipine (5 mg/kg) or MT-1207 (10 mg/kg). Systolic BP in the amlodipine and MT-1207 treatment groups decreased by more than 20 mmHg and remained stable at all examined time points over 1, 2, 3, and 4 months (Fig. 4f), which was significantly different from that of the vehicle control group. The heart rate of the MT-1207 group was stable after chronic treatment (Supplementary Information Fig. S4), with an average of 410 beats/min, and there was no significant difference between the MT-1207 group and vehicle group at any time point. However, the heart rate of the amlodipine group increased slightly in the first 51 days compared with the vehicle group and gradually returned to normal later.

Three months after the treatments, six rats died in the vehicle control group, one died in the amlodipine group, and two died in the MT-1207 group (Fig. 4g). Nearly 8 months after administration, all rats in the vehicle control group died, and only five rats in the amlodipine and MT-1207 groups died. The survival rate of SHR-SP in both treatment groups was significantly different from that of SHR-SP in the vehicle control group, indicating that amlodipine and MT-1207 had a significant effect on delaying the occurrence of stroke and improving the survival rate in SHR-SP.

Effect of MT-1207 on isolated vascular function
To observe the effects of MT-1207 on vascular function, isolated aortae were precontracted by various vasoconstrictors and then used to test whether MT-1207 has vasodilatory effects. MT-1207 produced dose-dependent relaxation in all precontracted aortae induced by all examined vasoconstrictors, including adrenaline (10^{-5} mol/L), KCl (60 mmol/L), noradrenaline (10^{-5} mol/L), and 5-HT (10^{-5} mol/L) (Fig. 5a–d). These results suggest an antihypertensive effect of MT-1207, at least through its direct action on blood vessels.

Study on the molecular targets of MT-1207 action
The activity of MT-1207 was evaluated with enzyme and radioligand binding assays. In the preliminary screening experiment, the binding inhibitory effect of MT-1207 at 1 μM was tested on 87 molecular targets that are potentially involved in the regulation of BP and vascular function (Fig. 6 and Supplementary Information Table S1). Among them, 17 targets entered the secondary screening, on which MT-1207 had more than 50% binding inhibitory activity. Further dose-response experiments involving radioligand binding assays using five concentrations of MT-1207 (0.1 nM, 1 nM, 10 nM, 0.1 μM, and 1 μM) showed that the binding inhibition activities of MT-1207 on three receptors, i.e., adrenergic α_{1A}, adrenergic α_{1B}, and 5-HT_{2A} were very high, with

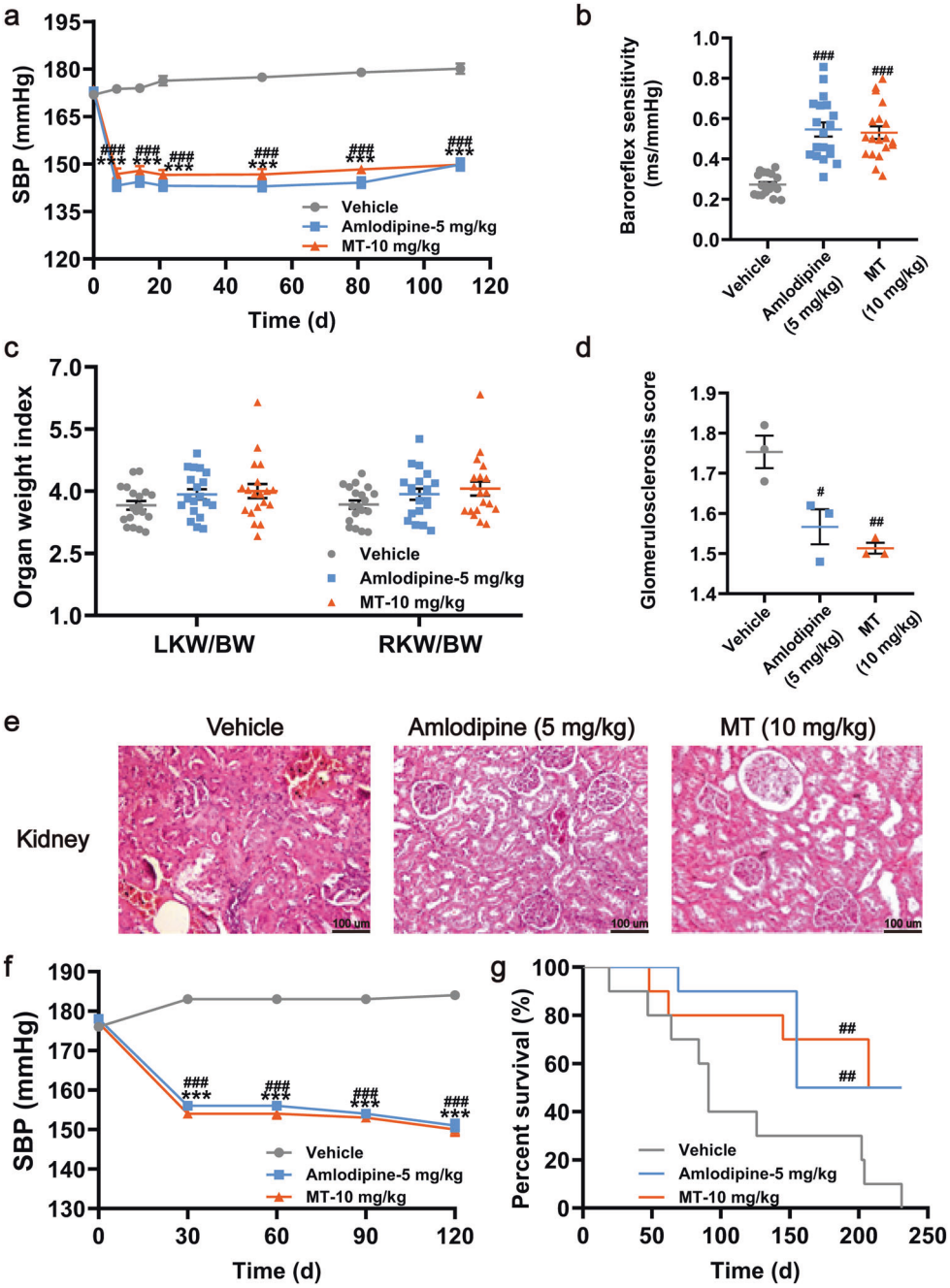

Fig. 4 Effects of long-term treatment with MT-1207 on spontaneously hypertensive rats (SHR) and stroke-prone spontaneously hypertensive rats (SHR-SP). Effects of long-term treatment with MT-1207 on systolic blood pressure (SBP) (**a**), baroreflex sensitivity (**b**), organ weight index (**c**) and glomerulosclerosis score (**d**) in SHR. **e** Representative renal histological changes in SHR after long-term treatment with amlodipine (5 mg · kg^{-1} · d^{-1}) and MT-1207 (10 mg · kg^{-1} · d^{-1}). Effects of long-term treatment with MT-1207 on SBP (**f**) and survival rate (**g**) in SHR-SP. MT, MT-1207. LKW left kidney weight, RKW right kidney weight, BW body weight. Data are shown as the mean ± SEM. $n = 20$ per group in SHR and $n = 10$ per group in SHR-SP. $^{***}P < 0.001$ versus day 0 (before drug administration). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ versus vehicle group

IC_{50} values less than 1 nM and K_i values less than 0.1 nM (Fig. 7). The binding inhibition activity of MT-1207 on another receptor, adrenergic α_{1D}, ranked fourth with an IC_{50} of 1.4 nM and a K_i value of 0.69 nM. Detailed parameters of IC_{50}, K_i and n_H values, as well as dose-response data for the activity of MT-1207 on 17 molecular targets, are listed (Supplementary Information Tables S2 and S3), and dose-response curves of MT-1207 on the inhibition of α_{1A}, α_{1B}, α_{1D}, and 5-HT_{2A} receptors are provided (Supplementary Information Fig. S5).

We further measured the antagonistic effect of MT-207 on α_1 and 5-HT receptors in isolated aortae. Phenylephrine, an α_1 receptor agonist, was used to generate a dose-response curve of vasoconstriction. As shown in Fig. 8a, MT-1207 at 3×10^{-8} mol/L shifted the dose-response curve of phenylephrine to the right, with the maximal response almost unchanged. The pA_2 value of MT-1207 against phenylephrine-induced contraction in aortae was 8.45 ± 0.03. Similarly, the dose-response curve of 5-HT was shifted to the right, with the maximal response almost unchanged after

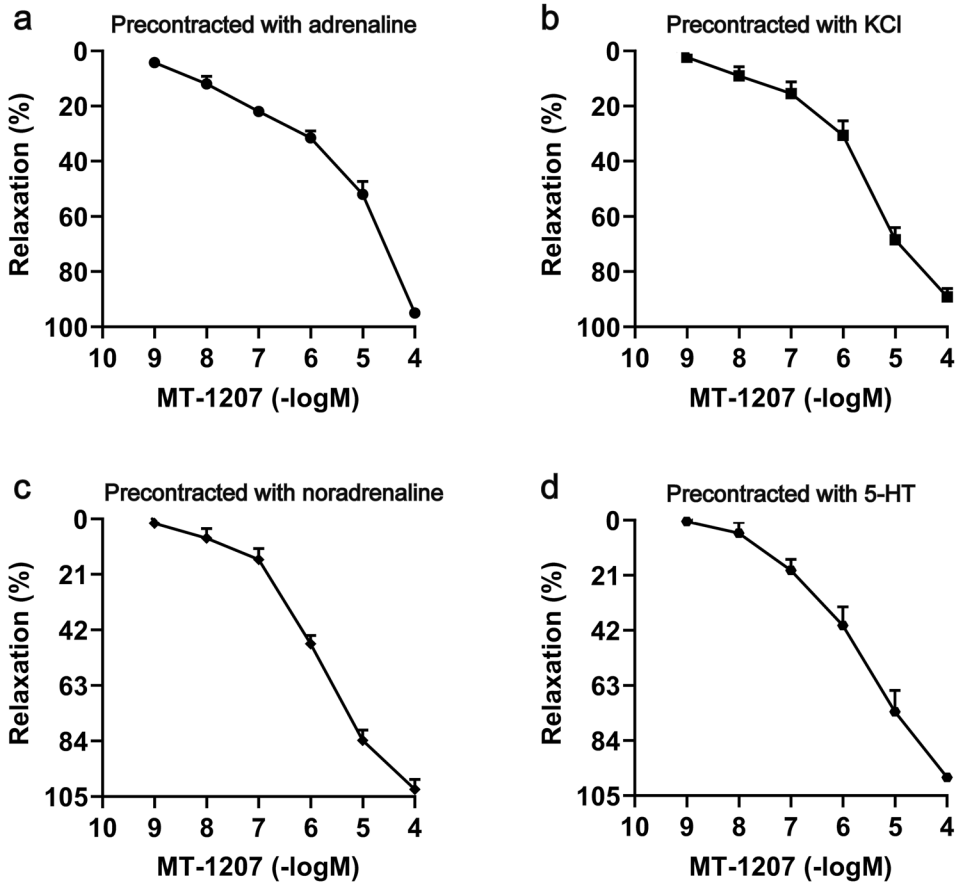

Fig. 5 Effects of MT-1207 on isolated aortic function. MT-1207 produced dose-dependent relaxation in precontracted aortic rings induced by adrenaline 10^{-5} mol/L (**a**), KCl 60 mmol/L (**b**), noradrenaline 10^{-5} mol/L (**c**), and 5-hydroxytryptamine (5-HT) 10^{-5} mol/L (**d**). Data are shown as the mean ± SEM. $n = 6$.

treatment with MT-1207 at 10^{-7} mol/L (Fig. 8b). MT-1207 antagonized the aortic contraction induced by 5-HT with a pA_2 value of 8.86 ± 0.14.

The results of isolated heart function are shown in Fig. 8c. MT-1207 dose-dependently reduced heart rate, and with increasing dose, the effect appeared earlier. MT-1207 at a dose of 0.15 μM had no direct effect on heart rate during 30 min of observation. However, heart rates in the MT-1207 groups at doses of 0.3, 0.6, and 1.2 μM were significantly decreased compared with those before drug administration (data for MT-1207 at 0.6 μM not shown due to too many curves in Fig. 8c).

To provide insight into the binding mode of compound MT-1207 with the 5-HT_{2A} receptor, we performed molecular docking studies based on the crystal structure of the 5-HT_{2A} receptor with the antipsychotics risperidone and zotepine (PDB ID: 6A93). As shown in Fig. 8d, the benzotriazole moiety of MT-1207 formed π–π stacking interactions with Phe340 and Trp336. In addition, hydrophobic interactions between the thiazolyl and piperazinyl moieties and the surrounding residues were observed.

DISCUSSION

The present study reveals that MT-1207 is a novel multitarget antihypertensive agent that reduces hypertensive organ damage and stroke mortality. The main findings include the following: (a) In both genetic and experimental hypertensive models, i.e., SHR and 2K1C dogs, single administration of MT-1207 intragastrically or by gavage produces an effective hypotensive effect in a dose-dependent manner. This hypotensive effect occurs very rapidly and without an increase in heart rate, which is in contrast with that of amlodipine, a commonly used antihypertensive drug. (b) In both SHR and 2K1C dogs, multiple administrations of MT-1207 by gavage for 1 week markedly decreased BP with a slight reduction in heart rate. After withdrawal of MT-1207, BP and heart rate returned to their levels observed before drug administration. (c) Long-term treatment of SHR with MT-1207 mixed in rat chow for 4 months and of stroke-prone SHR for ~8 months achieves an effective BP reduction and maintains a steady BP reduction at all examined time points, accompanied by improvement of BRS (an overall index of cardiovascular function), protection of renal and cardiovascular organs, and reduction in stroke mortality, which is similar to the effects of amlodipine. (d) MT-1207 has a high potency to bind and antagonize adrenergic α_{1A}, α_{1B}, and α_{1D}, and 5-HT_{2A} receptors, which cause vasorelaxation and slow heart rate, thus contributing to the hypotensive effect involved in the treatment of hypertension and related organ damage (Fig. 8e).

The current therapeutic goals for hypertension are to achieve effective BP reduction as well as obvious organ protection [1–3]. These goals were truly achieved by long-term treatment with MT-1207 in SHR and stroke-prone SHR. It seemed that effective and maximal BP reduction occurred within 1 week of MT-1207 treatment and was maintained at a steady state at the examined time points during long-term treatment with MT-1207. BRS is an overall index of cardiovascular function and may predict the outcome of cardiovascular disease [17, 18]. In animal studies, we have shown that impaired BRS causes cardiovascular and renal damage [13, 19–21], aggravates hypertensive organ damage and atherosclerosis [13, 22], and increases the mortality of stroke and myocardial infarction [23, 24]. We have also demonstrated that BRS is impaired in hypertension and has a negative relationship

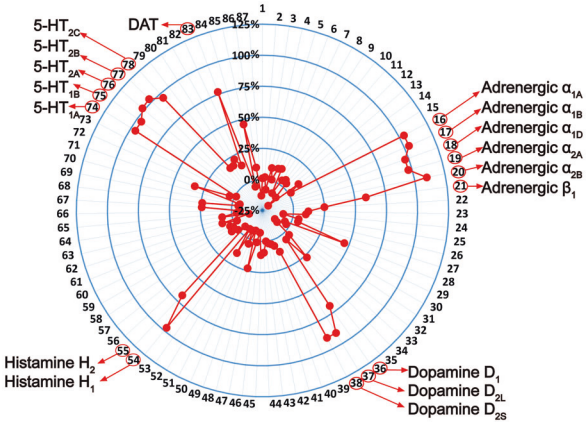

Fig. 6 Binding inhibition percentage of MT-1207 (1 μM) on 87 potential targets in primary screening. Each point represents the mean binding inhibition percentage from measurements in two individual experiments. The binding inhibition percentage increases from the center to the periphery of the circle. The 87 examined molecular targets in primary screening were as follows: (1) ATPase, Na^+/K^+, Heart; (2) Cholinesterase, Acetyl, ACES; (3) Cyclooxygenase COX-1; (4) Cyclooxygenase COX-2; (5) Monoamine Oxidase MAO-A; (6) Monoamine Oxidase MAO-B; (7) Peptidase, Angiotensin-Converting Enzyme; (8) Peptidase, CTSG (Cathepsin G); (9) Phosphodiesterase PDE3; (10) Phosphodiesterase PDE4; (11) Protein Serine/Threonine Kinase, PKC, Non-Selective; (12) Protein Tyrosine Kinase, Insulin Receptor; (13) Protein Tyrosine Kinase, LCK; (14) Adenosine A_1; (15) Adenosine A_{2A}; (16) Adrenergic α_{1A}; (17) Adrenergic α_{1B}; (18) Adrenergic α_{1D}; (19) Adrenergic α_{2A}; (20) Adrenergic α_{2B}; (21) Adrenergic β_1; (22) Adrenergic β_2; (23) Androgen (Testosterone); (24) Angiotensin AT_1; (25) Bradykinin B_2; (26) Calcium Channel L-Type, Benzothiazepine; (27) Calcium Channel L-Type, Dihydropyridine; (28) Calcium Channel L-Type, Phenylalkylamine; (29) Calcium Channel N-Type; (30) Cannabinoid CB_1; (31) Cannabinoid CB_2; (32) Chemokine CCR1; (33) Chemokine CXCR2 (IL-$8R_B$); (34) Cholecystokinin CCK_1 (CCK_A); (35) Cholecystokinin CCK_2 (CCK_B); (36) Dopamine D_1; (37) Dopamine D_{2L}; (38) Dopamine D_{2S}; (39) Endothelin ET_A; (40) Estrogen ERα; (41) $GABA_A$, Chloride Channel, TBOB; (42) $GABA_A$, Flunitrazepam, Central; (43) $GABA_A$, Ro-15-1788, Hippocampus; (44) $GABA_{B1A}$; (45) Glucocorticoid; (46) Glutamate, AMPA; (47) Glutamate, Kainate; (48) Glutamate, Metabotropic, $mGlu_5$; (49) Glutamate, NMDA, Agonism; (50) Glutamate, NMDA, Glycine; (51) Glutamate, NMDA, Phencyclidine; (52) Glutamate, NMDA, Polyamine; (53) Glycine, Strychnine-Sensitive; (54) Histamine H_1; (55) Histamine H_2; (56) Leukotriene, Cysteinyl $CysLT_1$; (57) Melanocortin MC_1; (58) Melanocortin MC_4; (59) Muscarinic M_1; (60) Muscarinic M_2; (61) Muscarinic M_3; (62) Muscarinic M_4; (63) Neuropeptide YY_1; (64) Nicotinic Acetylcholine; (65) Nicotinic Acetylcholine α1, Bungarotoxin; (66) Opiate δ_1 (OP1, DOP); (67) Opiate κ (OP2, KOP); (68) Opiate μ (OP3, MOP); (69) Platelet Activating Factor (PAF); (70) Potassium Channel [K_{ATP}]; (71) Potassium Channel hERG; (72) PPARγ; (73) Progesterone PR-B; (74) Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{1A}$; (75) Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{1B}$; (76) Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{2A}$; (77) Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{2B}$; (78) Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{2C}$; (79) Serotonin (5-Hydroxytryptamine) $5\text{-}HT_3$; (80) Sodium Channel, Site 2; (81) Tachykinin NK_1; (82) Transporter, Adenosine; (83) Transporter, Dopamine (DAT); (84) Transporter, GABA; (85) Transporter, Norepinephrine (NET); (86) Transporter, Serotonin (5-Hydroxytryptamine) (SERT); (87) Vasopressin V_{1A}. The 17 targets on which MT-1207 (1 μM) had more than 50% binding inhibition activities are indicated.

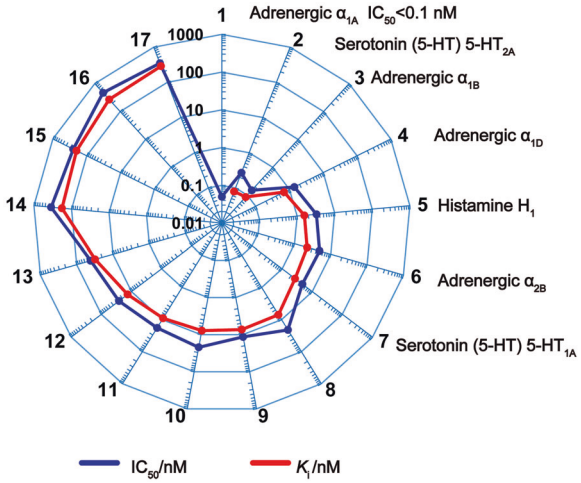

Fig. 7 IC_{50} and K_i for the inhibitory effect of MT-1207 on 17 molecular targets in secondary screening. Each point represents the mean IC_{50} (blue) or K_i (red) from determinations in two individual experiments. IC_{50} or K_i increases logarithmically moving from the center to the periphery of the circle. The 17 examined molecular targets in secondary screening were as follows: (1) Adrenergic α_{1A}; (2) Serotonin (5-HT) $5\text{-}HT_{2A}$; (3) Adrenergic α_{1B}; (4) Adrenergic α_{1D}; (5) Histamine H_1; (6) Adrenergic α_{2B}; (7) Serotonin (5-HT) $5\text{-}HT_{1A}$; (8) Dopamine D_{2L}; (9) Serotonin (5-HT) $5\text{-}HT_{2B}$; (10) Dopamine D_{2S}; (11) Adrenergic α_{2A}; (12) Serotonin (5-HT) $5\text{-}HT_{2C}$; (13) Serotonin (5-HT) $5\text{-}HT_{1B}$; (14) Dopamine D_1; (15) Transporter, Dopamine (DAT); (16) Adrenergic β_1; (17) Histamine H_2. Seven targets with IC_{50} and K_i values less than 10 nM are indicated.

with hypertensive organ damage and that recovery of BRS can improve hypertensive organ damage and the outcome of cardiovascular diseases [7, 8, 23, 25]. The present study showed a significant improvement in BRS after 4 months of MT-1207 treatment, indicating an overall improvement in cardiovascular system function [26]. Morphological examinations of the renal and cardiovascular systems (the kidney, heart, brain, and aorta) also showed significant protection against hypertensive organ damage, especially an obvious reduction in glomerular sclerosis score. To further assess the influence of long-term MT-1207 treatment on the outcome of hypertension and cardiovascular disease, stroke mortality was observed in a genetic strain of stroke-prone hypertensive rats, i.e., stroke-prone SHR. These animals die from ischemic stroke in the majority of cases and from hemorrhagic stroke in the minority of cases, as we previously described [27]. During ~8 months of experimental observation, stroke-prone SHR in the vehicle treatment group all died, whereas 50% of stroke-prone SHR survived with MT-1207 treatment, indicating that long-term MT-1207 treatment can reduce stroke mortality, thus improving the end points of hypertension.

Many antihypertensive drugs show a reflex increase in heart rate when they reduce BP, which may lead to cardiac side effects and weaken the hypotensive effect. MT-1207 had a rapid hypotensive effect within 5 min after drug administration and gastrointestinal absorption. Simultaneously, it did not induce reflex tachycardia in hypertensive rats and dogs. In fact, it showed a slight reduction in heart rate after both single and multiple administrations. This bradycardic effect of MT-1207 appears to be due to its direct effect on the heart. As seen from the results of isolated heart function in Fig. 8c, MT-1207 dose-dependently reduced heart rate, and as the dose increased, the effect appeared earlier. According to our preliminary pharmacokinetics study (data not shown), 0.3 μM was nearly equivalent to a single dose of 10 mg/kg in rats. Therefore, the results support that MT-1207 can directly inhibit heart rate. Molecular target studies demonstrated that MT-1207 antagonized the $5\text{-}HT_{2A}$ receptor. Existence of the $5\text{-}HT_{2A}$ receptor in the heart is well characterized, where it is known to increase heart rate [28, 29]. Thus, $5\text{-}HT_{2A}$ receptor blockade by MT-1207 directly inhibits the heart rate. In the second screening experiment of molecular target studies in vitro, we noticed that in addition to $5\text{-}HT_{2A}$, there are two other heart rate-related targets, adrenergic β_1 and histamine H_2 receptors. However, the IC_{50} of MT-1207 on adrenergic β_1 and histamine H_2 was 1740 and 1296

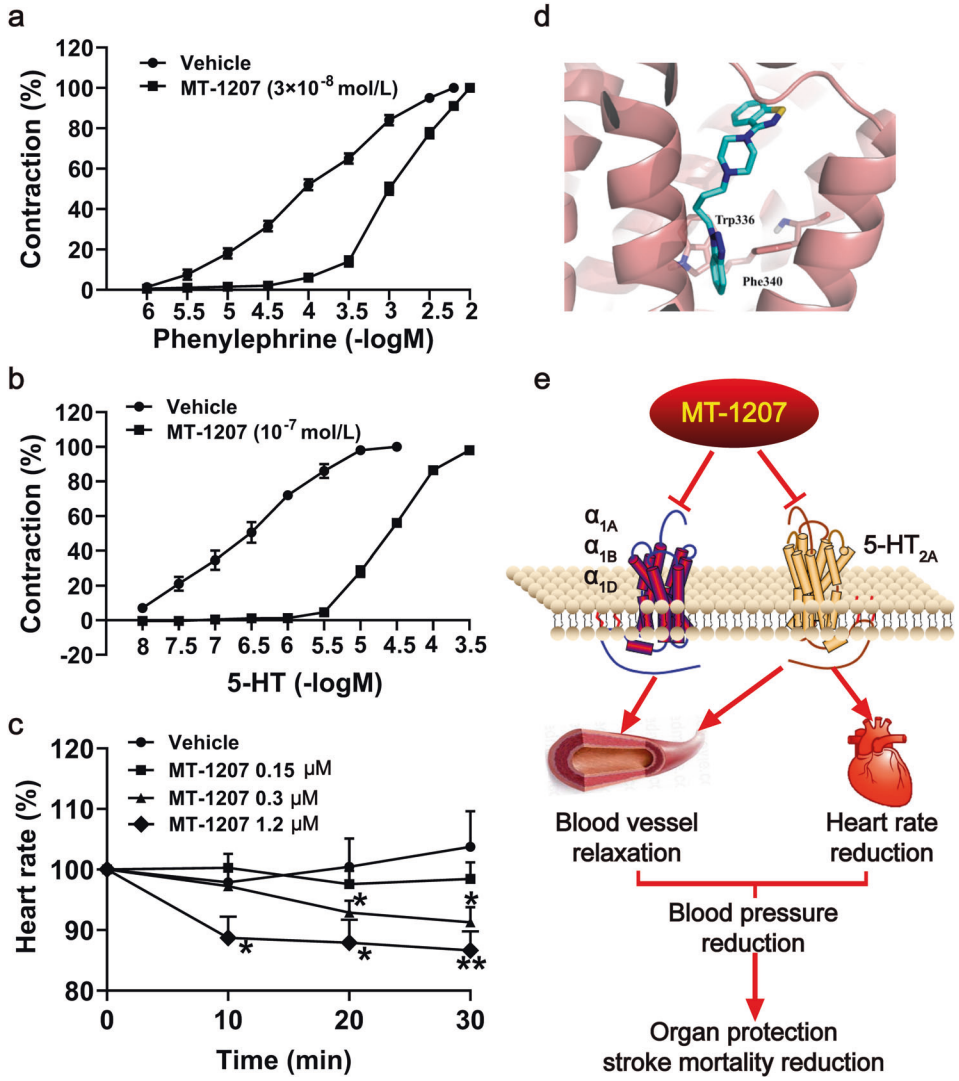

Fig. 8 Adrenergic α_{1A}, α_{1B}, and α_{1D}, and 5-HT_{2A} receptors mediate the antihypertensive effect of MT-1207. In isolated aortae, MT-1207 shifted the dose-response curves of phenylephrine (**a**) and 5-HT (**b**) to the right, with the maximal response unchanged. **c** Effect of MT-1207 on heart rate in isolated hearts. Data are shown as the mean ± SEM. $n = 6$ per group. $^*P < 0.05$, $^{**}P < 0.01$, versus min 0 (before drug administration). **d** Proposed binding mode of MT-1207 with 5-HT_{2A} (PDB ID: 6A93). **e** Proposed mechanism for MT-1207 as a novel multitarget antihypertensive agent.

times that of the 5-HT_{2A} receptor, respectively. Therefore, MT-1207 inhibits heart rate mainly by blocking the 5-HT_{2A} receptor. In addition, the effective concentration in vivo is usually higher than that in vitro. Species differences between in vitro screening and whole animal studies should also be considered. Thus, whether MT-1207 has an effect on adrenergic β_1 and histamine H_2 in vivo needs further study.

In isolated blood vessels, MT-1207 produced dose-dependent vasorelaxation in precontracted aortae induced by various vasoconstrictors, indicating a direct effect of MT-1207 on blood vessels. To study the involvement of calcium channels, we screened four types of calcium channels through radioligand binding experiments during primary screening. At a concentration of 1 μM, MT-1207 had no inhibitory effect on benzothiazine-sensitive L-type calcium channels, dihydropyridine-sensitive L-type calcium channels or N-type calcium channels but had a low inhibitory effect of 46% on L-type calcium channels sensitive to phenylalkylamine. Because the 0.3 μM drug plasma concentration was nearly equivalent to a single dose of 10 mg/kg MT-1207 in rats, as mentioned above, the blocking effect on calcium channels was relatively weak at this drug concentration in vivo.

In molecular target studies, MT-1207 strongly antagonized adrenergic α_{1A}, α_{1B}, and α_{1D}, and 5-HT_{2A} receptors. It is well characterized that these receptors in blood vessels mediate vasoconstriction [28]. Thus, the vasodilation of MT-1207 can be mediated by its blockade of adrenergic α_{1A}, α_{1B}, and α_{1D}, and 5-HT_{2A} receptors in blood vessels. Further studies in isolated blood vessels also showed that MT-1207 shifted the dose-response curves of aortic contractions induced by either α_1 agonist (phenylephrine) or 5-HT_2 agonist (5-HT) to the right. These inhibitory effects of MT-1207 on vasoconstriction further support that MT-1207 can produce vasodilation via adrenergic α_{1A}, α_{1B}, and α_{1D}, and 5-HT_{2A} receptors in blood vessels. Additionally, MT-1207 appeared only to shift the dose-response curves to the right but not to inhibit the maximal effects of both phenylephrine and 5-HT, indicating that MT-1207 is a competitive antagonist of adrenergic α_{1A}, α_{1B}, and α_{1D}, and 5-HT_{2A} receptors.

We also noticed that MT-1207 had a strong binding inhibition effect on some other targets, such as histamine H_1, adrenergic α_{2B}, and 5-HT_{1A} receptors. However, histamine H_1 can cause blood vessel dilation and has little effect on heart rate [30]. The α_2 receptor is mainly distributed in the presynaptic membrane of

noradrenergic nerves and has a BP-lowering effect [31]. 5-HT_{1A} is associated with a reduction in BP and heart rate [32]. Therefore, from the current pharmacodynamic experiments and target-binding experiments, MT-1207 plays a role in reducing BP mainly by antagonizing the 5-HT_{2A} receptor and α_1 receptor. Whether MT-1207 can exert antihypertensive effects through other receptors remains to be further confirmed by the combination of pharmacokinetic data and in vivo experiments. Whether inhibition of other targets produces noncardiovascular effects will also be investigated in future studies.

In a molecular modeling study, we further proposed a reasonable binding mode of MT-1207 with the 5-HT_{2A} receptor. However, the crystal structures of adrenergic α_{1A}, α_{1B}, and α_{1D} have not yet been reported, and it is hard to determine the binding mode of the remaining structure of MT-1207 with α_1 receptors. In this article, we mainly investigated the pharmacological effects and potential targets of MT-1207. The interaction mode between MT-1207 and targets will be further investigated in future crystal structure research.

Therefore, the mechanism underlying the hypotensive effect of MT-1207 is at least attributed to vasodilation via adrenergic α_{1A}, α_{1B}, and α_{1D} and 5-HT_{2A} receptors as well as bradycardia via the 5-HT_{2A} receptor. In molecular target studies, 87 molecular targets related to the regulation of BP were included for the primary screening and secondary verification. Some known targets for antihypertensive drugs, such as angiotensin-converting enzyme (ACE), angiotensin II type 1 receptor (AT_1), and ion channels of calcium (L-type) and potassium (K_{ATP}), appeared to not be involved in the hypotensive effect of MT-1207, since the effect of MT-1207 on these molecules was nonexistent or very weak. It was noted that the effect of MT-1207 on potassium channel hERG was also very weak, suggesting safety for the development of MT-1207 as an antihypertensive drug [33]. Detailed information regarding the effects of MT-1207 on 87 molecular targets listed in Supplementary Information Tables S1, S2, and S3 is valuable for further characterization of MT-1207 pharmacodynamics, in combination with its pharmacokinetics.

In summary, the present study characterizes the effectiveness and targets of MT-1207, demonstrating that MT-1207 is a novel and promising single-molecule multitarget agent for hypertension treatment that has entered into clinical trials in China. In a follow-up study, the molecular binding mode of MT-1207 with potential targets needs to be elucidated through crystal structure research. In addition, the antihypertensive mechanism of MT-1207 still needs more in vivo evidence. Given that a multitarget therapeutic strategy is essential for antihypertensive treatment and that single-molecule multitarget drugs are a trend for modern drug research and development, this study will promote further research on MT-1207 and its clinical trial progress.

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AUTHOR CONTRIBUTIONS

CYM, JGL, and DFS designed the study. TYX and CYM wrote the manuscript. TYX, PW, JST, SLQ, YHH, and JGL performed the experiments. CYM, JGL, TYX, PW, JST, SLQ, SNW, YHH, and JYX analyzed the data.

ADDITIONAL INFORMATION

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REFERENCES

1. Williams B, Mancia G, Spiering W, Agabiti Rosei E, Azizi M, Burnier M, et al. 2018 ESC/ESH Guidelines for the management of arterial hypertension: The Task Force for the management of arterial hypertension of the European Society of Cardiology and the European Society of Hypertension. J Hypertens. 2018;36:1953–2041.
2. Writing Group of 2018 Chinese Guidelines for the Management of Hypertension, Chinese Hypertension League, Chinese Society of Cardiology, Chinese Medical Doctor Association Hypertension Committee, Hypertension Branch of China International Exchange and Promotive Association for Medical and Health Care, Hypertension Branch of Chinese Geriatric Medical Association. et al. 2018 Chinese guidelines for the management of hypertension. Chin J Cardiovasc Med. 2019;24:1–42.
3. Su DF, Miao CY. Reduction of blood pressure variability: a new strategy for the treatment of hypertension. Trends Pharmacol Sci. 2005;26:388–90.
4. Miao CY. Cardio-cerebro-vascular pharmacology. Beijing (CHN): Science Press; 2019.
5. Forouzanfar MH, Liu P, Roth GA, Ng M, Biryukov S, Marczak L, et al. Global burden of hypertension and systolic blood pressure of at least 110 to 115 mmHg, 1990–2015. JAMA. 2017;317:165–82.
6. Miao CY, Zhu QY, Yang YC, Su DF. Antihypertensive effects of atenolol and nitrendipine alone or in combination on three hypertensive models of rats. Zhongguo Yao Li Xue Bao. 1992;13:448–51.
7. Xie HH, Miao CY, Jiang YY, Su DF. Synergism of atenolol and nitrendipine on hemodynamic amelioration and organ protection in hypertensive rats. J Hypertens. 2005;23:193–201.
8. Han P, Shen FM, Xie HH, Chen YY, Miao CY, Mehta JL, et al. The combination of atenolol and amlodipine is better than their monotherapy for preventing end-organ damage in different types of hypertension in rats. J Cell Mol Med. 2009;13:726–34.
9. Xu LP, Shen FM, Shu H, Miao CY, Jiang YY, Su DF. Synergism of atenolol and amlodipine on lowering and stabilizing blood pressure in spontaneously hypertensive rats. Fundam Clin Pharmacol. 2004;18:33–8.
10. Fu YL, Tao L, Peng FH, Zheng NZ, Lin Q, Cai SY, et al. GJA1-20k attenuates Ang II-induced pathological cardiac hypertrophy by regulating gap junction formation and mitochondrial function. Acta Pharmacol Sin. 2020. https://doi.org/10.1038/s41401-020-0459-6.
11. Ling G, Liu AJ, Shen FM, Cai GJ, Liu JG, Su DF. Effects of combination therapy with atenolol and amlodipine on blood pressure control and stroke prevention in stroke-prone spontaneously hypertensive rats. Acta Pharmacol Sin. 2007;28:1755–60.
12. Wu MY, Ma XJ, Yang C, Tao X, Liu AJ, Su DF, et al. Effects of allisartan, a new AT_1 receptor blocker, on blood pressure and end-organ damage in hypertensive animals. Acta Pharmacol Sin. 2009;30:307–13.
13. Miao CY, Xie HH, Zhan LS, Su DF. Blood pressure variability is more important than blood pressure level in determination of end-organ damage in rats. J Hypertens. 2006;24:1125–35.
14. Xu TY, Lan XH, Guan YF, Zhang SL, Wang X, Miao CY. Chronic nicotine treatment enhances vascular smooth muscle relaxation in rats. Acta Pharmacol Sin. 2015;36:429–39.
15. Kimura KT, Asada H, Inoue A, Kadji FMN, Im D, Mori C, et al. Structures of the 5-HT_{2A} receptor in complex with the antipsychotics risperidone and zotepine. Nat Struct Mol Biol. 2019;26:121–8.
16. Jones G, Willett P, Glen RC, Leach AR, Taylor R. Development and validation of a genetic algorithm for flexible docking. J Mol Biol. 1997;267:727–48.
17. La Rovere MT, Bigger JT Jr., Marcus FI, Mortara A, Schwartz PJ. Baroreflex sensitivity and heart-rate variability in prediction of total cardiac mortality after myocardial infarction. ATRAMI (Autonomic Tone and Reflexes After Myocardial Infarction) Investigators. Lancet. 1998;351:478–84.
18. La Rovere MT, Pinna GD, Hohnloser SH, Marcus FI, Mortara A, Nohara R, et al. Baroreflex sensitivity and heart rate variability in the identification of patients at risk for life-threatening arrhythmias: implications for clinical trials. Circulation. 2001;103:2072–7.
19. Miao CY, Su DF. The importance of blood pressure variability in rat aortic and left ventricular hypertrophy produced by sinoaortic denervation. J Hypertens. 2002;20:1865–72.
20. Miao CY, Su DF. Arterial baroreflex function and left ventricular hypertrophy. Drug Dev Res. 2003;58:61–4.
21. Miao CY, Tao X, Gong K, Zhang SH, Chu ZX, Su DF. Arterial remodeling in chronic sinoaortic-denervated rats. J Cardiovasc Pharmacol. 2001;37:6–15.
22. Cai GJ, Miao CY, Xie HH, Lu LH, Su DF. Arterial baroreflex dysfunction promotes atherosclerosis in rats. Atherosclerosis. 2005;183:41–7.
23. Liu AJ, Ma XJ, Shen FM, Liu JG, Chen H, Su DF. Arterial baroreflex: a novel target for preventing stroke in rat hypertension. Stroke. 2007;38:1916–23.
24. Yu JG, Song SW, Shu H, Fan SJ, Liu AJ, Liu C, et al. Baroreflex deficiency hampers angiogenesis after myocardial infarction via acetylcholine-alpha7-nicotinic ACh receptor in rats. Eur Heart J. 2013;34:2412–20.

25. Lu ZA, Xie HH, Xu LP, Yin AF, Miao CY, Su DF. Restoration of arterial baroreflex function contributes to organ protection in spontaneously hypertensive rats treated with long-term hydrochlorothiazide mixture. Clin Exp Pharmacol Physiol. 2003;30:49–54.
26. Li Y, Feng Y, Liu L, Li X, Li XY, Sun X, et al. The baroreflex afferent pathway plays a critical role in H_2S-mediated autonomic control of blood pressure regulation under physiological and hypertensive conditions. Acta Pharmacol Sin. 2020. https://doi.org/10.1038/s41401-020-00549-5.
27. Zhang W, Liu AJ, Yi-Ming W, Liu JG, Shen FM, Su DF. Pressor and non-pressor effects of sodium loading on stroke in stroke-prone spontaneously hypertensive rats. Clin Exp Pharmacol Physiol. 2008;35:83–8.
28. Alexander SPH, Christopoulos A, Davenport AP, Kelly E, Mathie A, Peters JA, et al. THE CONCISE GUIDE TO PHARMACOLOGY 2019/20: G protein-coupled receptors. Br J Pharmacol. 2019;176:S21–S141.
29. Miao CY, Su DF. Negative chronotropic effect of ketanserin on the rat right atria and mechanism analysis. J Sec Milit Med Univ. 1990;11:397–400.
30. Panula P, Chazot PL, Cowart M, Gutzmer R, Leurs R, Liu WL, et al. International Union of Basic and Clinical Pharmacology. XCVIII. Histamine receptors. Pharmacol Rev. 2015;67:601–55.
31. Taylor BN, Cassagnol M. Alpha Adrenergic Receptors. Treasure Island (FL): StatPearls Publishing; 2021.
32. Barnes NM, Ahern GP, Becamel C, Bockaert J, Camilleri M, Chaumont-Dubel S, et al. International Union of Basic and Clinical Pharmacology. CX. Classification of receptors for 5-hydroxytryptamine; pharmacology and function. Pharmacol Rev. 2021;73:310–520.
33. Garrido A, Lepailleur A, Mignani SM, Dallemagne P, Rochais C. hERG toxicity assessment: useful guidelines for drug design. Eur J Med Chem. 2020;195:112290.