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RESEARCH ARTICLE

Identification and characterization of the new generation soluble guanylate cyclase stimulator BAY-747 designed for the treatment of resistant hypertension

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Abstract

Background and Purpose: First-generation soluble guanylate cyclase (sGC) stimulators have shown clinical benefit in pulmonary hypertension (riociguat) and chronic heart failure (vericiguat). However, given the broad therapeutic opportunities for sGC stimulators, tailored molecules for distinct indications are required.

Experimental Approach: We report the high-throughput screening (HTS)-based discovery of a second generation of sGC stimulators from a novel imidazo[1,2-*a*]pyridine lead series. An intense medicinal chemistry programme resulted in the discovery of the sGC stimulator BAY 1165747 (BAY-747). The pharmacokinetic profile of BAY-747 was determined in different species, and it was broadly characterized in pharmacological model systems relevant for vasodilatation and hypertension.

Key Results: BAY-747 is a highly potent sGC stimulator in vitro. In addition, BAY-747 showed an excellent pharmacokinetic profile with long half-life and low peak-to-trough ratio. BAY-747 was investigated in experimental *in vivo* models of malignant and resistant hypertension (rHT). In spontaneously hypertensive (SH) rats, BAY-747 caused a dose-related and long-lasting decrease in mean arterial blood pressure (MAP). Oral treatment over 12 days resulted in a persistent decrease. BAY-747 provided additional benefit when dosed on top of losartan, amlodipine or spironolactone and even on top of triple combinations of frequently used antihypertensive drugs. In a new canine model of rHT, BAY-747 caused a dose-related and long-lasting (>6 h) MAP decrease.

Conclusion and Implications: BAY-747 is a potent, orally available sGC stimulator. BAY-747 shows long-acting pharmacodynamic effects with a very low peak-to-

Abbreviations: BAY-747, BAY 1165747; CTEPH, chronic thromboembolic pulmonary hypertension; HFrEF/HFpEF, chronic heart failure with reduced/preserved ejection fraction; MAP, mean arterial blood pressure; PAH, pulmonary arterial hypertension; PK, pharmacokinetic; rHT, resistant hypertension; SCD, sickle cell disease; uHTS, ultra-high-throughput screening.

trough ratio. BAY-747 could be a treatment alternative for patients with hypertension, especially those not responding to standard-of-care therapy.

KEYWORDS
blood pressure, HTS, resistant hypertension, sGC stimulator, soluble guanylate cyclase

1 | INTRODUCTION

Soluble guanylate cyclase (sGC) is attracting rapidly growing interest as a therapeutic target in cardiopulmonary, cardiovascular (CV) and cardiorenal diseases. In 2013 and 2021, the first sGC stimulators, riociguat (Adempas®) and vericiguat (Verquvo®), were approved for the treatment of pulmonary arterial hypertension (PAH), chronic thromboembolic pulmonary hypertension (CTEPH) and chronic heart failure with reduced ejection fraction (HFrEF), respectively (Sandner et al., 2021). In addition, the sGC stimulator praliciguat was in clinical development for chronic diabetic kidney disease (Hanrahan et al., 2020) but seems—to our knowledge—currently to be on hold for this indication. Thus, this first generation of sGC stimulators represents a very effective treatment approach in the field of pulmonology, cardiology, nephrology and even beyond (Buys et al., 2018; Friebe et al., 2020). This broad therapeutic use is based on the unique mode of action of sGC stimulators, which can restore the pivotal nitric oxide (NO)–sGC–guanosine 3′,5′-cyclic monophosphate (cGMP) pathway in a broad variety of cells and tissues. Binding of sGC stimulators to sGC triggers the formation of cGMP independently of endogenous NO. In addition, sGC stimulators work synergistically with even very low concentrations of endogenous NO (Sandner et al., 2021).

Because NO production is impaired or very much down-regulated in many disease states, this independent and synergistic mode of action of sGC stimulators might explain the broad treatment potential. NO-derived intracellular cGMP is also a powerful vasodilatory mediator, and in fact, sGC stimulators have demonstrated dose-dependent and pronounced blood pressure (BP)-lowering effects in various experimental models of hypertension (HT) (Follmann et al., 2017; Mittendorf et al., 2009; Stasch, Dembowsky, et al., 2002). This is of particular interest because, despite various treatment approaches including combination treatment for hypertensive patients, a significant proportion of patients do not or at least do not adequately respond to antihypertensive therapy.

This therapy-refractory, resistant HT (rHT) is defined as BP that remains above goal in spite of compliance with optimal doses of ≥3 antihypertensive agents of different classes ideally including a diuretic (Carey et al., 2018; Mancia et al., 2007). The worldwide number of patients with HT increased substantially in recent years, from 2.18 billion in 1990 to 4.06 billion in 2020 (Brant et al., 2022). There is also a dramatic increase of the number of patients in developing countries, low-income countries and in Asia, driven by the adoption of Western lifestyle resulting in obesity, renal insufficiency and diabetes (Bromfield & Munter, 2013; Mills et al., 2020). Among all these HT patients, 15% to 20% have uncontrolled and treatment-resistant HT, which worsens CV and cardiorenal outcomes and also decreases life expectancy mainly by increasing CV death (Achelrod et al., 2015; Brant et al., 2022; Carey et al., 2019). Despite focused efforts and various combination therapies, there is a high medical need for more effective treatment options. Therefore, it is an intriguing concept that active vasodilation through sGC stimulation could add an additional mode of action to established antihypertensive therapies, which mainly block vasoconstriction, such as beta-blockers and calcium antagonists. An sGC stimulator could be highly effective in rHT either as stand-alone therapy or when combined with existing therapy that mainly blocks the renin–angiotensin–aldosterone system (RAAS) pathway.

However, sGC stimulators differ in potency, selectivity and also in their pharmacokinetic (PK) profile and tissue exposure. Especially for the treatment of HT, ideal sGC stimulators should have a long-lasting duration of action suitable for once-daily dosing and low peak-to-trough ratios in order to maintain BP control over 24 h. Therefore, our goal was to design novel sGC stimulators fulfilling these properties.

What is already known

- Among hypertensive patients, 15% to 20% have uncontrolled and treatment-resistant hypertension.
- sGC stimulators have pronounced blood pressure-lowering effects in various experimental models of hypertension.

What does this study add

- BAY-747 belongs to a new chemical class of long-acting sGC stimulators.
- BAY-747 dosing on top of triple combinations of frequently used antihypertensive drugs provides additional benefit.

What is the clinical significance

- The sGC stimulator BAY-747 provides a potentially effective strategy for patients with therapy-resistant hypertension.

We describe here the ultra-high-throughput screening (uHTS)-based identification and characterization of BAY 1165747 (BAY-747), which belongs to a new chemical class of long-acting sGC stimulators. We pharmacologically characterized BAY-747 in vitro as well as *in vivo*. BAY-747 shows a typical sGC stimulator profile and could substantially reduce BP, even when given on top of combinations of standard of care. In addition, the PK profile of BAY-747 is unique and might be optimal for the treatment of HT and rHT.

2 | METHODS

2.1 | Cell-based sGC assay

High-throughput screening (HTS)-based identification and characterization of BAY-747 were performed using a rat sGC Chinese hamster ovary (CHO) (RRID:CVCL_0213) reporter cell line generated as described previously (Wunder et al., 2005). Laboratory compound testing was performed on 384-well microtitre plates (MTPs), and uHTS was performed on 1536-well MTPs. Cells were cultured for 1 day. After removal of the cell culture medium, cells were loaded for 3 h with coelenterazine at 37°C and 5% CO_2 in the absence or presence of 100-μM 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one (ODQ). Test compounds were added for 6 min. Luminescence measurements were performed using a charge-coupled device (CCD) camera (Hamamatsu, Hamamatsu, Shizuoka, Japan) in a light tight box. Data in Figure 2 are presented as mean values with standard deviation (SD) from a single experiment performed in quadruplicate.

2.2 | Isolated, purified sGC

The sGC was highly purified from a baculovirus/Sf9 expression system, and the effects of BAY-747 on enzyme activity were measured as described (Follmann et al., 2017). Enzyme activity was measured by the formation of [^{32}P]-cGMP from α-[^{32}P]-GTP. For enzyme characterization, the specific activity of sGC was expressed as x-fold stimulation versus specific basal activity.

2.3 | Isolated rabbit aortic rings

Animal studies are reported in compliance with the ARRIVE guidelines (Percie du Sert et al., 2020) and with the recommendations made by the *British Journal of Pharmacology* (Lilley et al., 2020). The relaxing effect of BAY-747 on phenylephrine-precontracted rabbit aortic rings was determined as previously described (Stasch, Alonso-Alija, et al., 2002). Chinchilla rabbits of either sex (Charles River Laboratories, Research Models and Services, Sulzfeld, Germany, about 2–3 kg) were killed by an overdose of thiopental (100 mg·kg^{-1}, i.v.). To assure death, cardiac arrest was verified. The aorta was dissected, and aortic rings (1.5-mm width) were suspended under an initial tension of approximately 4 g in 5-ml organ baths containing Krebs–Henseleit solution (containing 0.001% bovine serum albumin [BSA]) at 37°C. Contractions were measured isometrically with Statham UC2 strain gauges connected to a DAS1802HC data acquisition board (Keithley Instruments, Germering, Germany). Rings were precontracted by 3×10^{-8}-g·ml^{-1} phenylephrine (submaximal contraction) four times. Each contraction was followed by a series of 16 washing cycles and a resting period of 28 min. The test compound was added to the organ baths at the beginning of the last resting period. Rings were subsequently contracted by phenylephrine (3×10^{-8} g·ml^{-1}). Result is given as mean value ± standard error of the mean (SEM) (n = 7).

2.4 | Langendorff heart

Effects of BAY-747 on isolated-perfused rat Langendorff hearts were determined as previously described (Follmann et al., 2017). Male Wistar rats (Harlan NL, 200–250 g) were anaesthetized using Narcoren (pentobarbitone 100 mg·kg^{-1}, i.p.). The heart was rapidly excised and connected to a Langendorff perfusion system (FMI GmbH, Seeheim-Jugenheim, Germany). The heart was perfused at a constant rate of 10 ml·min^{-1} with Krebs–Henseleit bicarbonate buffer solution equilibrated with 95% O_2–5% CO_2. The perfusion solution contained (in mM) the following: NaCl 118, KCl 3, $NaHCO_3$ 22, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 1.8, glucose 10 and Na pyruvate 2. A pressure transducer registered the perfusion pressure in the perfusion system. Left ventricular pressure (LVP) was measured using a second pressure transducer connected to a water-filled balloon, which was inserted into the left ventricle via the left atrium. The end-diastolic pressure was initially set to 8 mmHg by adjusting the volume of the balloon. The hearts were spontaneously beating. The signals from the pressure transducer were amplified and registered by a personal computer (PowerLab Chart software, ADInstruments, Spechbach, Germany).

A 10-mM stock solution of BAY-747 was prepared in dimethyl sulfoxide (DMSO). BAY-747 (final concentration of DMSO: 0.01%) was added to the perfusion solution for 20 min at increasing concentrations ranging from 1 nM to 10 μM without wash-out between concentrations. During this time, the values reached steady state. The perfusion pressure before compound application was 141.9 ± 3.2 mmHg for the treated group and 137.9 ± 9.9 mmHg for the vehicle group. Values are presented as relative changes ± SEM (n = 5) of baseline values before compound application.

2.5 | Pharmacokinetics (PKs) *in vivo*

For *in vivo* PK experiments, male Wistar rats (Harlan NL, Horst, The Netherlands, 220–250 g) and female Beagle dogs (Marshall BioResources, North Rose, NY, USA, 8.5–10 kg) were used. PK parameters of BAY-747 were determined as described in Follmann et al. (2017). The PK parameters clearance (CL), terminal half-life ($t_{1/2}$), volume of distribution at steady state (V_{SS}) and bioavailability after oral administration (*F*)

were calculated from the plasma concentration − time course by using a validated in house PK calculation software (KinEx).

2.6 | Telemetered spontaneously hypertensive (SH) rats

BP and heart rate (HR) were monitored in freely moving conscious SH rats by radiotelemetry as described previously (Hahn et al., 2021). The SH rat was used as this is a well-established model of genetic essential HT. Female adult SH rats (Charles River, Germany, 200–250 g) were equipped with implantable radiotelemetry, and a data acquisition system (Data Sciences, St. Paul, MN, USA) comprising a chronically implantable transducer/transmitter unit equipped with a fluid-filled catheter was used. The telemetric system is composed of three basic elements: implantable transmitters (TA11PA-C40), receivers (RA1010) and a computer-based acquisition software (Dataquest A.R.T. 4.0 for Windows, DSI, St. Paul, MN, USA). The hardware configuration was equipped for 24 animals. Each rat cage was positioned on top of an individual receiver platform. The transmitter was implanted into the peritoneal cavity, and the sensing catheter was inserted into the descending aorta. All surgeries were performed under aseptic conditions in deep anaesthesia (isoflurane), and for postsurgical protection against infection and pain, Oxytetracyclin (Oxytetracyclin® 10%, 60 mg·kg^{-1}, s.c., Beta-Pharma GmbH & Co, Augsburg, Germany) and Rimadyl (Rimadyl®, 4 mg·kg^{-1}, s.c., Pfizer, Freiburg, Germany) were used, respectively. The rats fully recovered before being used for BP measurements. All animals were housed in individual cages, maintained on a 12-h light/dark cycle with free access to standard laboratory rat chow and water ad libitum. All test compounds including BAY-747 were prepared freshly before administration in 10% Transcutol (2-(2-ethoxyethoxy)ethanol), 20% Cremophor (polyethoxylated castor oil) and 70% de-ionised water and were applied orally by gavage. Application volume was always 2-ml·kg^{-1} body weight. The group size for the experiments using telemetered SH rats usually was n = 6. However, due to the fact that some animals died shortly before the start of the experiments and could not be replaced in due time, n = 5 was used for two dosing groups (Figure 5: BAY-747, 1 mg·kg^{-1}; Figure 8: BAY-747, 3 mg·kg^{-1}).

2.7 | Renin transgenic (RenTG) hypertensive rats treated with *Nω*-nitro-L-arginine–methylester (L-NAME)

Male RenTG rats carrying an additional mouse renin gene (RenTG (mRRen2)27) at the age of 8 weeks (breeding facility Bayer AG, Wuppertal, Germany) were used for these experiments. L-NAME was administered via the drinking water (50 mg·L^{-1}) in order to block NO synthesis and induce an endothelial dysfunction in all study groups. BAY-747 was administered by gavage at doses of 0.3 and 3 mg·kg^{-1}. BP was measured via the tail-cuff method before the start of the study (Day 0) to exclude pre-existing differences between the groups, and on Day 7.

2.8 | Telemetered hypertensive dogs

The influence of BAY-747 on BP and HR was studied in the hypertensive 'renal wrap' dog model as recently published (Vogel et al., 2021). This animal model was chosen as it replicates important features of human-resistant HT. Briefly, telemetry devices (Model L21, Data Sciences International (DSI), St. Paul, MN, USA) were implanted in five Beagle dogs for continuous BP and electrocardiogram (ECG) measurement. Subsequently, an rHT phenotype was established by unilateral renal wrapping combined with an occlusion of the contralateral renal artery.

For all surgery procedures (telemetry device implantation, renal wrapping and renal artery occlusion), animals were anaesthetized with thiopental sodium (Trapanal®, 0.25–0.5 mg·kg^{-1}, Byk Gulden, Konstanz, Germany) and pancuronium bromide (0.20- to 0.25-mg·kg^{-1} Pancuronium Inresa, Inresa Arzneimittel GmbH, Freiburg, Germany). All dogs were mechanically ventilated with O_2/N_2O (1:3). Anaesthesia was maintained with isoflurane (1% to 2% Isoflurane Baxter, Baxter, Halle, Germany). For analgesia, fentanyl (10–40 μg·kg^{-1}·h^{-1}, Mallinckrodt Inc., Webster Groves, MO, USA) was infused. In a first intervention, a telemetry device was implanted. After left-side thoracotomy, a pressure sensor catheter (Model L21, Data Sciences International, USA) was implanted in the aorta. For ECG measurements, electrodes were positioned directly on the heart. The electronic part of the device was implanted on the left thorax side. After implantation, the skin and muscle were closed by VICRYL suture (Ethicon, Johnson & Johnson, New Brunswig, NJ, USA). All animals received enteral antibiotic (clindamycin [Cleorobe®], Zoetis, Berlin, Germany; 150 mg per animal; p.o.) and carprofen (Rimadyl®, p.o., 50 mg per animal, Zoetis, Germany) over a period of 10 days. For additional analgesia, a Durogesic® patch (fentanyl, 25 μg·h^{-1}, Janssen-Cilag, Neuss, Germany) was placed on the thorax side.

After wound healing, the left kidney was wrapped with silk in a second surgical procedure. Under sterile conditions, the left abdominal cavity was opened. The kidney was wrapped with sterilized silk. Afterwards, the peritoneum and all layers above, including the skin, were sutured using VICRYL suture (Ethicon, Johnson & Johnson, USA). Medication after the surgical intervention was applied as follows: For antibiotic prophylaxis, enrofloxacin (Baytril® 5 mg·kg^{-1}, Bayer Vital, Leverkusen, Germany) was injected subcutaneously the first day and was administered orally (Baytril Flavour, 50 mg per 10 kg, Bayer Vital, Germany) for the following 14 days. For analgesia, fentanyl patches (Durogesic®, 25-μg·h^{-1} fentanyl, Janssen-Cilag, Germany) were applied for three postsurgical days. Additionally, metamizole sodium (50 mg·kg^{-1}, Metamizol WDT, Wirtschaftsgenossenschaft deutscher Tierärzte eG, Garbsen, Germany) was administered intramuscularly on Day 1. Eight weeks after kidney wrapping, the contralateral (right) renal main artery was occluded by the insertion of a vascular plug® (Amplatzer Vascular Plug II, St. Jude Medical, St. Paul, MN, USA). The right common carotid artery was prepared for catheter intervention, and a vascular plug® was placed in the right renal artery to induce renal artery stenosis.

After development of stable HT conditions, different dosages of BAY-747 (0.05, 0.25, 0.75 and 1.25 mg·kg^{-1}) were then tested against placebo after oral application for their HR and BP effects. Furthermore, the highest dose of 1.25 mg·kg^{-1} was tested as repetitive applications on three consecutive days. Dosing was performed at 2:00 PM for all experiments.

2.9 | Preparation of compounds

BAY-747 (*N*-(2-amino-2-methylbutyl)-8-[(2,6-difluorobenzyl)oxy]-2,6-dimethylimidazo[1,2-*a*]pyridine-3-carboxamide) was synthesized by Bayer medicinal chemistry (Vakalopoulos et al., 2014). Chemical optimization of the screening hit to BAY-747 is described in detail in an accompanying paper (Vakalopoulos et al., 2023).

For in vitro experiments, a 10-mM DMSO solution was prepared and diluted in the respective aqueous buffers. For *in vivo* experiments, BAY-747 was solved in 10% Transcutol (2-(2-ethoxyethoxy)ethanol), 20% Cremophor (polyethoxylated castor oil) and 70% de-ionised water.

2.10 | Statistics

The data and statistical analysis comply with the recommendations of the *British Journal of Pharmacology* on experimental design and analysis in pharmacology (Curtis et al., 2022).

Curve fitting and calculation of IC_{50}/EC_{50} values were performed using GraphPad Prism software (Version 8, GraphPad Software Inc., San Diego, CA, USA).

The statistical analyses were performed using GraphPad Prism software (Version 9). All data were expressed as mean ± SEM or SD. Statistical significance was evaluated using the unpaired *t* test for two-group analysis. When comparing means from more than two groups, one-way analysis of variance (ANOVA) was corrected for multiple comparisons using Dunnett's test. Post-hoc tests were run only if F achieved P<0.05 and there was no significant variance inhomogeneity. For analysis of drug-related BP and HR effects, values were averaged between 1 and 7 h (Figures 4 and 6–8) or 0 and 12 days (Figure 5) after application and group differences were analysed. Differences were considered statistically significant at a level of probability smaller than 0.05 (*P < 0.05). The exact P level is not stated.

The sample sizes in the individual studies are based on long-term experience in characterizing test compounds or standard-of-care medications in these well-established models. The specific sample size used in each individual study can be found in the legend of the respective graphs. In the rodent studies, animals were randomized to individual treatment groups. This does not apply to the dog study. Here, all animals received all drug treatments in a cross-over designed study.

2.11 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org and are permanently archived in the Concise Guide to PHARMACOLOGY 2021/22 (Alexander et al., 2021).

3 | RESULTS

3.1 | Identification of a novel sGC stimulator class by uHTS

A cell-based uHTS study was performed using a recombinant rat sGC reporter cell line (Wunder et al., 2005) and Bayer's steadily growing compound library, including about 700,000 compounds from the former Schering AG. These compounds were tested in an automated uHTS assay using 1536-well MTPs. Confirmation of 321 primary hits in the cell-based and biochemical sGC assays led to the identification of a novel sGC stimulator imidazopyridine class, exemplified by the HTS hit shown in Figure 1. Intense chemical optimization resulted in the identification of the development candidate BAY-747. This novel imidazopyridine class represents the first potent sGC stimulator class discovered by uHTS.

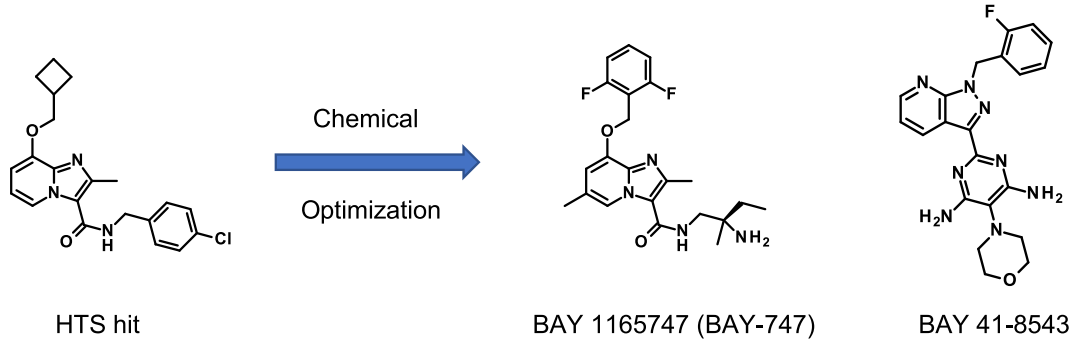

FIGURE 1 Structures of the high-throughput screening (HTS) hit and of the development candidate BAY 1165747 (BAY-747). The structure of BAY 41-8543, a member of the pyrazolopyrimidine class of soluble guanylate cyclase stimulators, is shown for comparison.

3.2 | Pharmacological characterization of BAY-747 in vitro

BAY-747 was examined on the stable sGC reporter cell line overexpressing rat sGC and stimulated the sGC reporter cell line with an EC_{50} value of 392 ± 259 nM (mean ± SD; n = 7). In the presence of the NO donor S–Nitroso–N–Acetyl–D,L–Penicillamine (SNAP, 30 and 100 nM), the EC_{50} value was shifted to 87 ± 28 and 33 ± 12 nM (mean ± SD; n = 5), respectively (Figure 2a). Pre-treatment of the sGC reporter cell line with 100μM ODQ for 3 h resulted in a reduced efficacy for BAY-747, and an EC_{50} value of 267 ± 173 nM (mean ± SD; n = 5; n.s.) was observed (Figure 2b). In addition, after ODQ pre-treatment, the NO donor SNAP (30 and 100 nM) had no effect on BAY-747-stimulated luminescence signals. We also tested effects of BAY-747 in the presence of the first-generation sGC stimulator BAY 41-8543 on our sGC reporter cell line. Addition of increasing concentrations of BAY-747 to the BAY 41-8543 concentration–response curve resulted in additive luminescence signals. Results are shown in Figure S1.

Next, we tested the effects of BAY-747 on highly purified rat sGC. BAY-747 stimulated the recombinant sGC from 0.01 to 100 μM in a concentration-dependent manner with shifts of 3-fold to 162-fold (Figure 2c). In addition, the sGC stimulatory effects of BAY-747 and the NO-releasing drug diethylamine NONOate (DEA/NO) alone and in combination were investigated. In combination, BAY-747 and DEA/NO synergized over a wide range of concentrations. At the highest concentrations of BAY-747 (100 μM) and DEA/NO (0.01 μM), the specific activity of sGC was 376-fold above baseline. The sGC stimulation induced by BAY-747 was nearly completely blocked by the sGC inhibitor ODQ and nearly absent in haem-free preparations.

Therefore, BAY-747 directly stimulates sGC by an NO-independent, but haem-dependent mechanism and strongly synergizes with NO. In conclusion, BAY-747 shows a pharmacological profile similar to previously identified sGC stimulators like riociguat and vericiguat (Follmann et al., 2017; Mittendorf et al., 2009).

The effect of BAY-747 on rabbit aortic rings pre-constricted with phenylephrine was also examined. BAY-747 concentration-dependently inhibited phenylephrine-induced contractions of rabbit aortic rings with an IC_{50} value of 27 ± 7 nM (Figure 3a).

In the isolated rat heart Langendorff preparation, BAY-747 reduced the coronary perfusion pressure in a concentration-dependent manner from 1 nM to 10 μM with a maximal effect of about 50% at the highest concentration (Figure 3b). BAY-747 up to

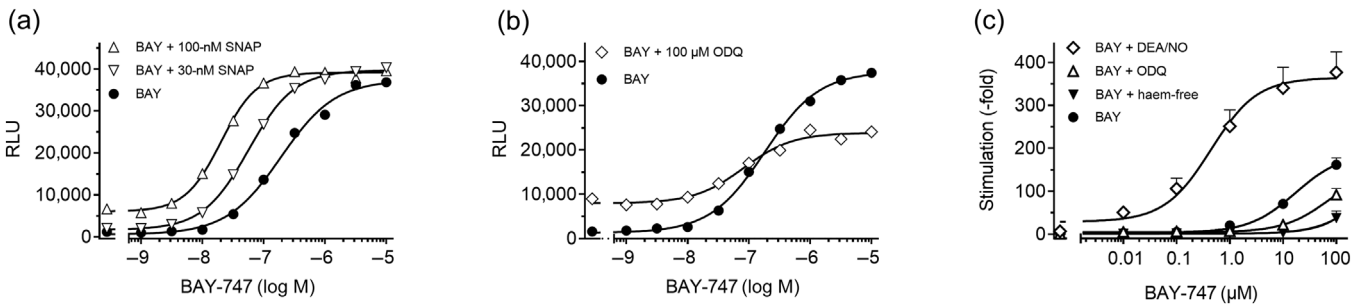

FIGURE 2 Effects of BAY-747 on the soluble guanylate cyclase (sGC) reporter cell line in the absence or presence of the nitric oxide donor SNAP (a) or after ODQ pre-treatment (b, c) on isolated highly purified sGC. Data in (a) and (b) are from a single experiment (performed in quadruplicate), representative of (a) 7 or (b) 5 experiments. DEA/NO, diethylamine NONOate; RLU, relative light units.

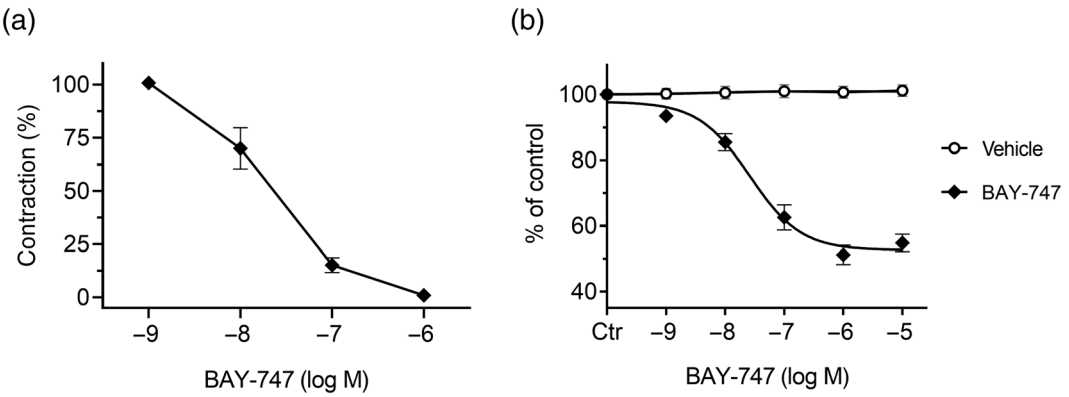

FIGURE 3 Vasorelaxing effects of BAY-747 on isolated vessels (a) and on perfusion pressure of isolated-perfused rat heart (b). Values are presented as relative changes ± standard error of the mean (a: n = 7; b: n = 6) of baseline values before compound application.

the highest concentration tested had no effect on HR, left ventricular diastolic pressure (LVDP) and contractility (+*dp*/*dt*).

In summary, the results of the in vitro pharmacological characterization consistently demonstrated that BAY-747 is a highly potent sGC stimulator. However, as a potential treatment option for broad CV indications such as HT, the PK profile of BAY-747 is of key importance. Therefore, BAY-747 was intensively investigated with respect to its PK profile in rats and dogs.

3.3 | Pharmacokinetics (PKs) in rat and dog

Intravenous PKs of BAY-747 were determined in Wistar rat and Beagle dog. In rat, low blood clearance (0.9 $L \cdot h^{-1} \cdot kg^{-1}$), high V_{SS} (12 $L \cdot kg^{-1}$) and a long terminal half-life of 5.9 h were observed. In dog, high blood clearance (1.8 $L \cdot h^{-1} \cdot kg^{-1}$), high V_{SS} (27 $L \cdot kg^{-1}$) and a long terminal half-life of 6.6 h were found. PK parameters were also determined after oral administration of BAY-747 in rat (see Figure S2) and dog. A high bioavailability was observed in rat (78%). Despite the high clearance in dog, a moderate bioavailability of 50% was observed.

Single-species scaling in rat predicted excellent human PKs (low clearance and long terminal half-life of >20 h) with a once-daily regime and a low efficacious dose. Irrespective of the high blood clearance in dog, single-species scaling using that species predicted a moderate human terminal half-life (>10 h) due to the high volume of distribution.

In order to support the use of BAY-747 for the treatment of rHT, a broad pharmacodynamic (PD) profiling of BAY-747 in rats and dogs in models with malignant HT and rHT was performed.

3.4 | Conscious SH rats

BAY-747 caused a dose-dependent and long-lasting decrease in mean arterial BP (MAP) in telemetered, conscious SH rats. A dose of 0.1 $mg \cdot kg^{-1}$ (not shown) had no substantial effect on BP. Oral administration of 0.3- to 3-$mg \cdot kg^{-1}$ BAY-747, however, lowered BP in a dose related fashion by 10%–30% (0.3 $mg \cdot kg^{-1}$: −14.8 ± 9.9 mmHg, n.s.; 1.0 $mg \cdot kg^{-1}$: −27.5 ± 5.7 mmHg, $P < 0.05$; 3.0 $mg \cdot kg^{-1}$: −37.6 ± 16.4 mmHg, $P < 0.05$; Figure 4). The peak effects occurred within the initial 2 h. The depressor effect plateaued for five to six additional hours at a dose of 0.3 $mg \cdot kg^{-1}$. After application of 1 and 3 $mg \cdot kg^{-1}$, there was an extended phase of BP reduction, which lasted for more than 24 h, accompanied by a dose-related transient tachycardia. HR increases observed were moderate to mild, between 15% and 30%, with increasing doses. Tachycardia subsided within 24 h.

Repeated treatment with BAY-747 over a period of 12 consecutive days at once-daily doses of 1 and 3 $mg \cdot kg^{-1}$, p.o., resulted in a persistent decrease in MAP (1.0 $mg \cdot kg^{-1}$: −43.5 ± 11.6 mmHg, $P < 0.05$; 3.0 $mg \cdot kg^{-1}$: −44.5 ± 24.6 mmHg, $P < 0.05$; Figure 5). BP reduction lasted for more than 24 h, depending on the dosage applied. A transient moderate HR increase was observed following the first three to four administrations. This initial tachycardia completely disappeared during the treatment period. Noteworthy, the extent of the antihypertensive effects was maintained over the period of treatment.

3.5 | Combination studies in SH rats

As pointed out in Section 1, guideline-based combination therapies are the gold standard of difficult to treat hypertensive patients. Therefore, the influence of BAY-747 on BP in combination with different antihypertensive medications was studied in telemetered SH rats (Figure 6). The effects of BAY-747 were studied in the presence of maximal tolerated doses of losartan (100 $mg \cdot kg^{-1}$, p.o., Figure 6a) and amlodipine (10 $mg \cdot kg^{-1}$, p.o., Figure 6b). Oral application of BAY-747 at doses of 3 and 1 $mg \cdot kg^{-1}$ caused an additional significant ($P < 0.05$) and long-lasting decrease in MAP when given on top of the maximal tolerated doses of the respective antihypertensive medication. A concomitant HR increase (reflex tachycardia) was observed in combination with amlodipine and losartan. BAY-747 dosing on top of losartan and amlodipine was adjusted to achieve maximal BP reductions of no more than 60 mmHg to avoid life-threatening hypotension.

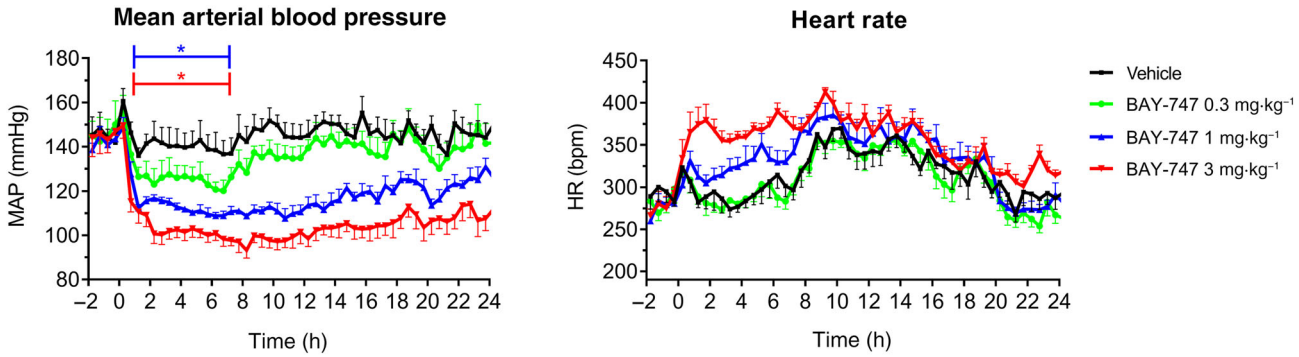

FIGURE 4 Effects of single-dose administration of BAY-747 (0.3–3 $mg \cdot kg^{-1}$, p.o.) on mean arterial blood pressure (MAP) and heart rate (HR) in conscious spontaneously hypertensive rats. Data (mean of n = 6 ± standard error of the mean) were analysed by one-way analysis of variance and multiple comparison tests (*$P < 0.05$, in comparison to vehicle group).

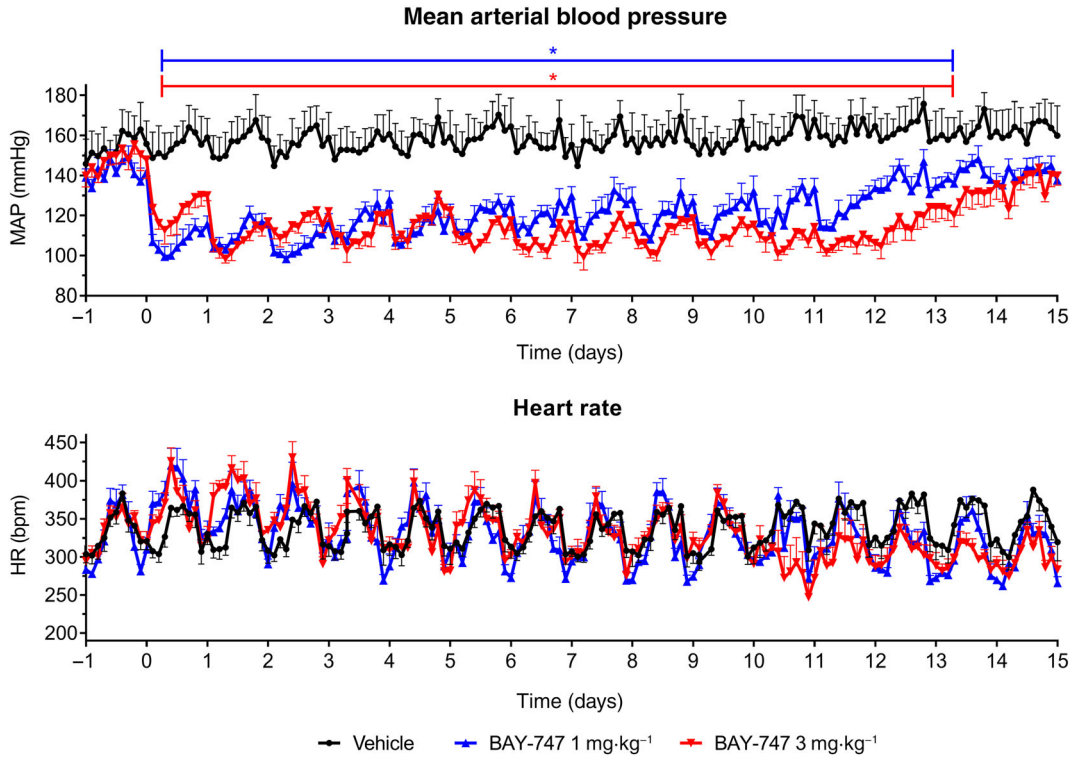

FIGURE 5 Effects on mean arterial blood pressure (MAP) and heart rate (HR) of repeated treatment with BAY-747 (1 and 3 mg·kg^{-1}, p.o.) in conscious spontaneously hypertensive rats over a period of 12 consecutive days. Data (mean of n = 5–6 ± standard error of the mean) were analysed by one-way analysis of variance and multiple comparison tests (*P < 0.05, in comparison to vehicle group).

BAY-747 was also characterized when administered on top of two different antihypertensive triple combinations (Figure 7). Either amlodipine (3 mg·kg^{-1}, p.o., triple combi 1) or atenolol (10 mg·kg^{-1}, p.o., triple combi 2) was used in combination with hydrochlorothiazide (30 mg·kg^{-1}, p.o.) and lisinopril (10 mg·kg^{-1}, p.o.). Oral application of BAY-747 at doses of 0.3 and 1 mg·kg^{-1} caused an additional, long-lasting decrease in MAP when given on top of the maximal tolerated doses of the respective antihypertensive triple medication (P < 0.05). BAY-747 dosing on top of the two triple medications was adjusted to achieve maximal BP reductions of no more than 60 mmHg to avoid life-threatening hypotension. Besides its BP-lowering effect, BAY-747 also induced an additional HR increase (reflex tachycardia) when given on top of the triple combination of amlodipine, hydrochlorothiazide and lisinopril (Figure 7a). The HR increase was not observed in the triple combination experiment using atenolol, hydrochlorothiazide and lisinopril (Figure 7b), presumably due to the presence of the beta-blocker atenolol.

3.6 | Combination study with spironolactone

Because spironolactone is an approved therapy for rHT, we tested the effects of BAY-747 on top of spironolactone. The influence of BAY-747 on BP in combination with spironolactone was studied in telemetered SH rats (Figure 8). Spironolactone was orally administered repeatedly at a dose of 30 mg·kg^{-1}, b.i.d., on four consecutive days. A slowly developing BP-lowering effect of spironolactone became visible over the treatment period of 4 days.

The effects of BAY-747 on top of spironolactone were studied on Day 4. BAY-747 at doses of 0.3 and 3 mg·kg^{-1} caused an additional, persistent decrease in MAP (0.3 mg·kg^{-1}: −12.4 ± 6.1 mmHg, n.s.; 3.0 mg·kg^{-1}: −25.8 ± 6.7 mmHg, P < 0.05) when given on top of spironolactone. BP reduction by BAY-747 lasted for more than 24 h, depending on the dosage applied. In addition, upon application of BAY-747, a concomitant HR increase (reflex tachycardia) was observed.

3.7 | Effects on BP in hypertensive RenTG rats under blockade of NO synthesis

To further assess the antihypertensive effects of BAY-747 in a situation in which endogenous NO is very low or absent, we performed an additional *in vivo* study in a hypertensive rat model in which NO synthesis was blocked. To this end, we treated hypertensive RenTG rats with the NO-synthase blocker L-NAME, which abrogates NO production. BAY-747 was administered in L-NAME-treated RenTG rats by gavage at doses of 0.3 and 3 mg·kg^{-1}. BP was measured via the tail-cuff method once before the start of the study (Day 0) to exclude pre-existing differences between the groups, and on Day 7. The sGC stimulator BAY-747 was able to reduce BP from 210 ± 4 mmHg in the L-NAME-treated vehicle group to 199 ± 9 mmHg (n.s.) and 158 ± 4 mmHg (P < 0.05) after treatment with 0.3- and 3-mg·kg^{-1} BAY-747, respectively (Figure 9).

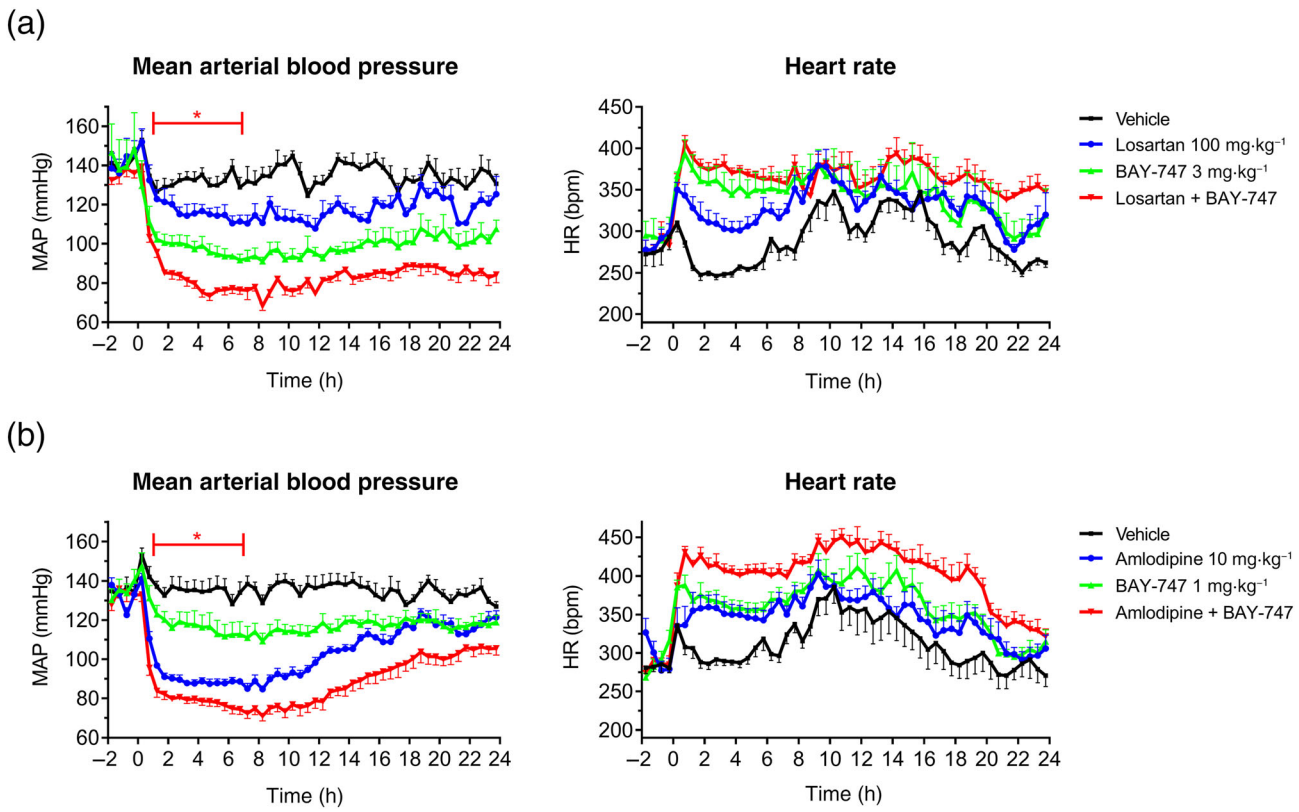

FIGURE 6 Effects of BAY-747 (3 mg·kg^{-1} [a] and 1 mg·kg^{-1} [b], respectively) on mean arterial blood pressure (MAP) and heart rate (HR) in conscious spontaneously hypertensive rats when given in combination with (a) losartan (100 mg·kg^{-1}, p.o.) or (b) amlodipine (10 mg·kg^{-1}, p.o.). Data (mean of n = 6 ± standard error of the mean) were analysed by one-way analysis of variance and multiple comparison tests (*P < 0.05, drug combination compared to SoC alone).

3.8 | Hypertensive renal wrap dogs

We also wanted to see if BAY-747 is effective in non-rodent species and in large animal models characterized by rHT. Therefore, the influence of BAY-747 on BP and HR was also studied in the hypertensive 'renal wrap' dog model. After telemetry device implantation, rHT was generated by wrapping one kidney with silk combined with an occlusion of the main kidney artery at the contralateral side (Vogel et al., 2021). Approximately 4 weeks after the interventions, the dogs developed a stable HT.

The effects of BAY-747 were studied after oral administration of 0.05- to 1.25-mg·kg^{-1} BAY-747 to awake, telemetered hypertensive dogs, beginning 6 weeks after rHT induction. At dose of ≤0.25 mg·kg^{-1}, no substantial effects on BP and HR were observed. BAY-747 at doses of 0.75 and 1.25 mg·kg^{-1} caused a robust (0.75 mg·kg^{-1}: −27.1 ± 2.0 mmHg; 1.25 mg·kg^{-1}: −22.0 ± 5.6 mmHg) and long-lasting (>6 h) decrease in MAP and a concomitant rise in HR (Figure 10a). Consistent effects on both systolic and diastolic pressures were observed. However, a trend towards a more pronounced decrease in systolic values compared to diastolic values was observed after treatment with BAY-747 (data not shown). The peak effects occurred within the initial 2 h after application of the compound. Only a limited number of n = 4 animals with intact telemetry signals were available for these studies. Therefore, no statistical analysis was done. Three times consecutive daily administration of 1.25-mg·kg^{-1} BAY-747 did not reveal signs of accumulative effects or the development of tachyphylaxis (Figure 10b).

4 | DISCUSSION

With increasing evidence for implication in various pathological processes, sGC stimulation has been identified as a promising therapeutic target in a growing number of human diseases. By their unique mode of action, sGC stimulators might also be ideal for the treatment of HT, especially for rHT when other mechanisms have failed (Buys & Sips, 2014; Chrysant, 2021; Franco & Oparil, 2006).

To identify new sGC stimulators with different physicochemical profiles, we screened the Bayer compound library and identified a novel imidazopyridine class of sGC stimulators representing the first example of a successful identification of a novel sGC stimulator lead series by uHTS.

Chemical optimization succeeded in the identification of BAY-747 as a candidate for further preclinical and clinical development. This new class of imidazopyridines differs from previous class of sGC stimulators like riociguat and vericiguat in terms of chemical structure and physicochemical properties. Most importantly, BAY-747 exhibits high water solubility (approx. 700 mg·L^{-1}, in buffer at pH = 6.5) mediated by its novel core and its amine substitution in the periphery.

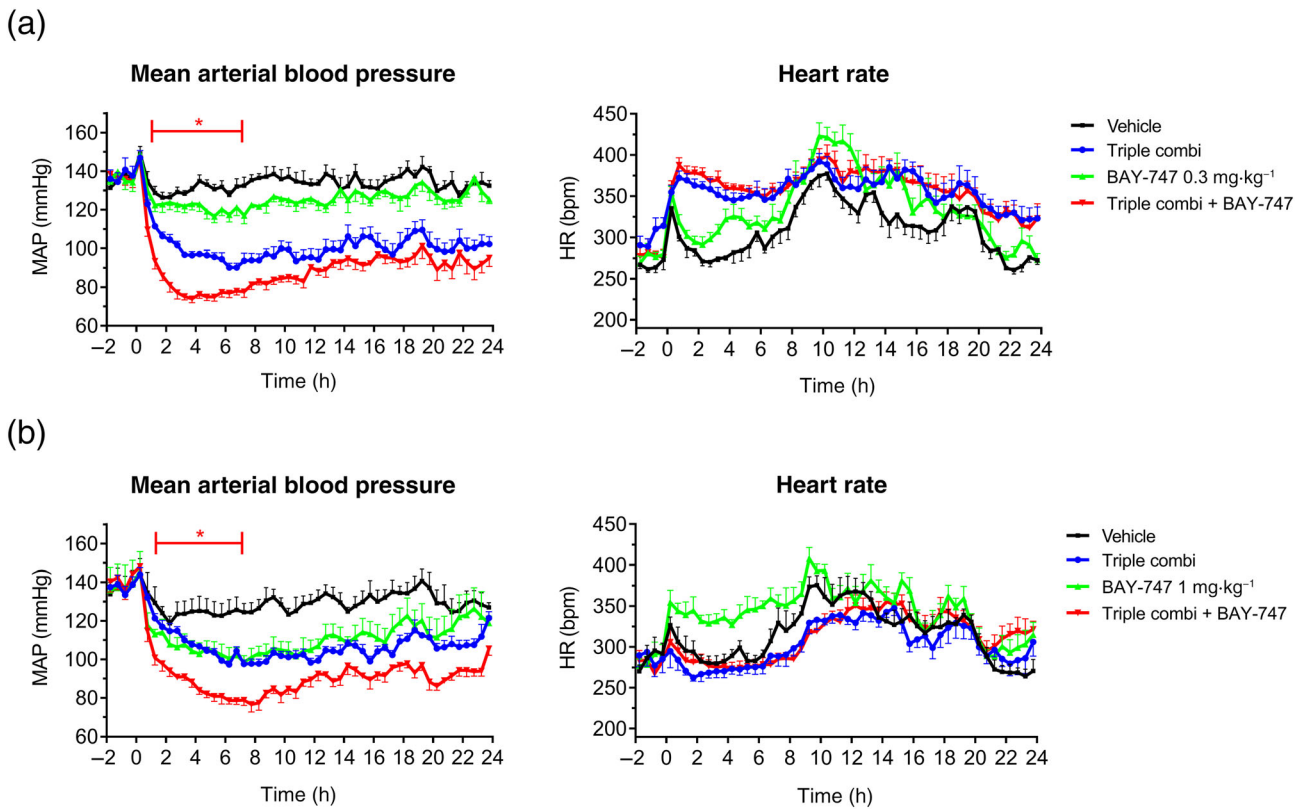

FIGURE 7 Effects of BAY-747 (0.3 or 1 mg·kg^{-1}, p.o.) on mean arterial blood pressure (MAP) and heart rate (HR) in conscious spontaneously hypertensive rats when given on top of triple combinations (combi) of hydrochlorothiazide (30 mg·kg^{-1}, p.o.), lisinopril (10 mg·kg^{-1}, p.o.) and (a) amlodipine (3 mg·kg^{-1}, p.o.) or (b) atenolol (10 mg·kg^{-1}, p.o.). Data (mean of n = 6 ± standard error of the mean) were analysed by one-way analysis of variance and multiple comparison tests (*P < 0.05, BAY-747 on top of triple combination compared to triple combination).

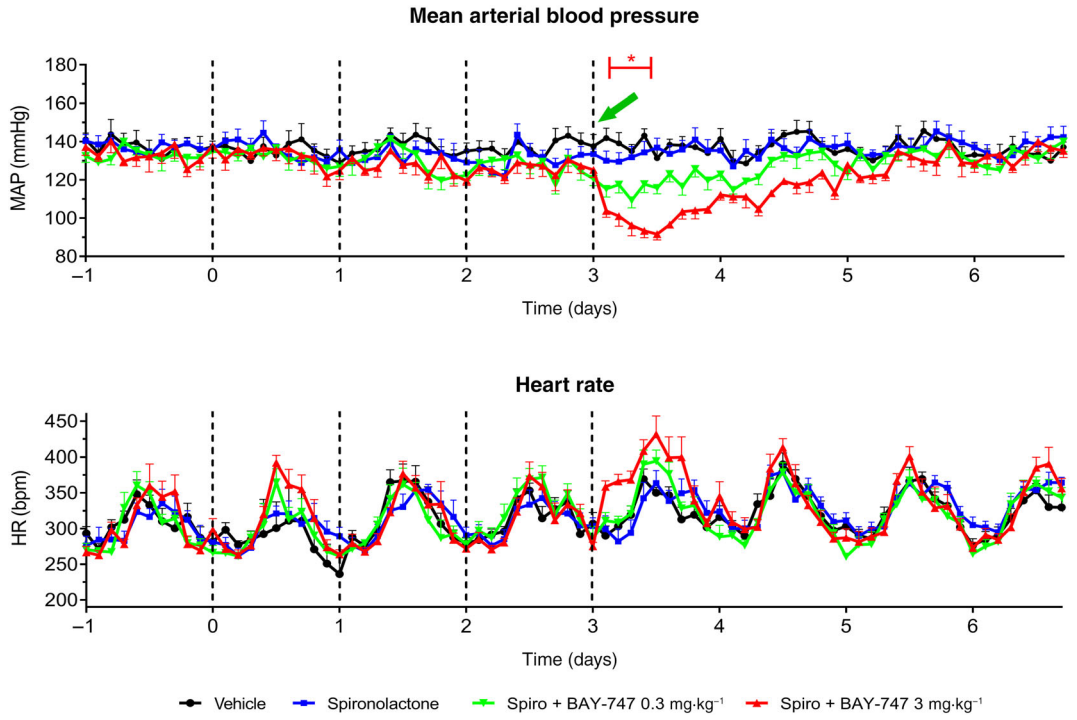

FIGURE 8 Effects of BAY-747 (0.3 or 3 mg·kg^{-1}, p.o.) on mean arterial blood pressure (MAP) and heart rate (HR) in conscious spontaneously hypertensive rats when given in combination with spironolactone (Spiro) (30 mg·kg^{-1}, p.o. b.i.d.). BAY-747 was given on Day 4 (arrow). Data (mean of n = 5–6 ± standard error of the mean) were analysed by one-way analysis of variance and multiple comparison tests (*P < 0.05, BAY-747—3.0 mg·kg^{-1} on top of Spiro compared to Spiro alone).

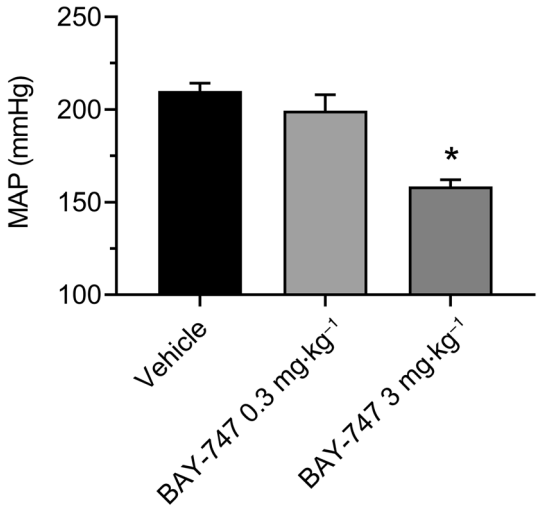

FIGURE 9 Effects of BAY-747 on mean arterial blood pressure (MAP) in hypertensive transgenic (mREN2)27 rats pre-treated for 7 days with N^{ω}-nitro-L-arginine methyl ester (L-NAME). The soluble guanylate cyclase stimulator BAY-747 was able to reduce blood pressure from 210 ± 4 mmHg in the L-NAME-treated vehicle group to 199 ± 9 mmHg (n.s.) and 158 ± 4 mmHg (*P < 0.05, in comparison to vehicle group) after treatment with 0.3- and 3-mg·kg^{-1} BAY-747, respectively. Data (mean of n = 8–12 ± standard error of the mean) were analysed by one-way analysis of variance and multiple comparison tests.

We thoroughly characterized the novel, chemically optimized sGC stimulator BAY-747 in vitro as well as *in vivo*. BAY-747 shows a typical sGC stimulator profile in vitro leading to an increase in cGMP in an NO-independent manner but also enhancing the effect of NO in vitro (Figure 2). In addition, BAY-747 shows additive effects when tested in the presence of the first-generation sGC stimulator BAY 41-8543. The results imply that first- and second-generation sGC stimulators bind to the same binding site on sGC. However, this has to be further elucidated by future studies, for example, by cryo-electron microscopy. In addition, BAY-747 concentration-dependently relaxed coronary blood vessels in the ex vivo Langendorff heart (Figure 3). In vivo, BAY-747 lowered BP in rats and dogs in a highly effective and dose-related manner.

Up to now, sGC stimulators have been approved for the treatment of patients with PAH, CTEPH (riociguat) and HFrEF (vericiguat) and were also clinically investigated in chronic heart failure with preserved ejection fraction (HFpEF), sickle cell disease (SCD) and fibrotic diseases. However, less is known about their safety and effectiveness in patients with HT and especially rHT. So far, only preliminary studies have been performed and have shown that the administration of sGC stimulators resulted in significant reductions of BP in both patients with HT and in subjects with normal BP, which was also seen in normotensive and hypertensive animal models (Chrysant, 2021; Sharovska et al., 2010; Stasch et al., 2011).

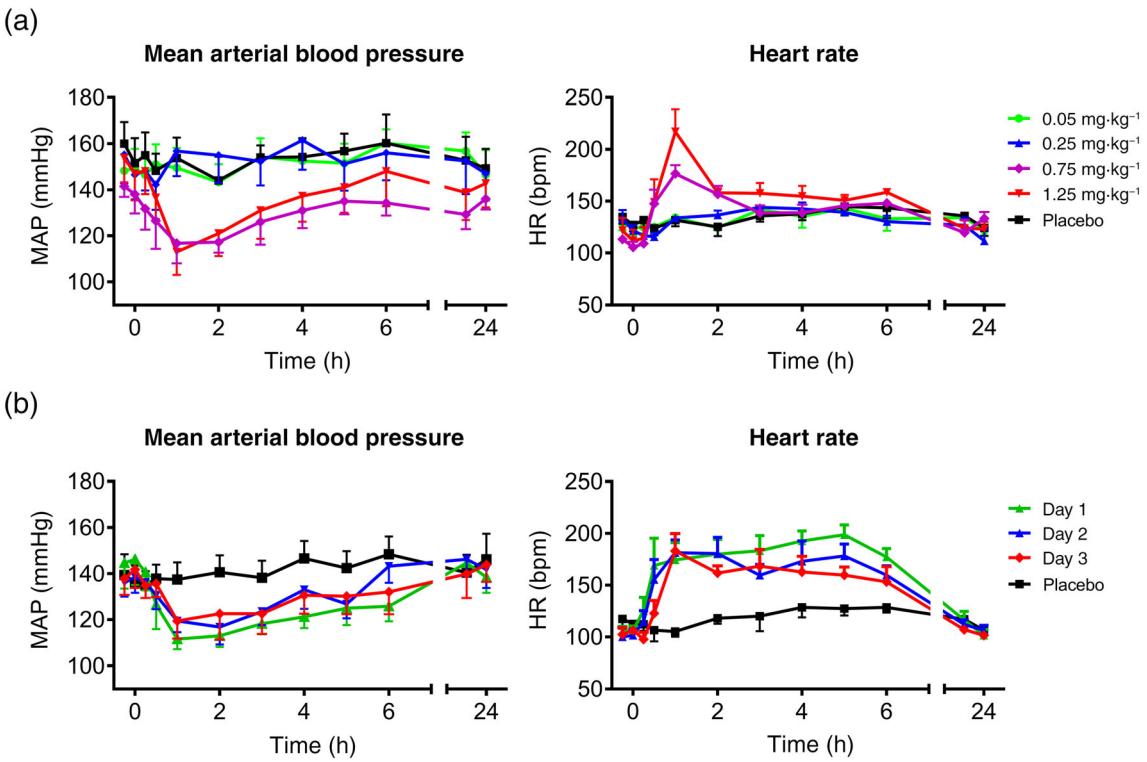

FIGURE 10 Effect of (a) single application and (b) repeated applications (1.25 mg·kg^{-1} each) of BAY-747 on mean arterial blood pressure (MAP) and heart rate (HR) in hypertensive dogs. Data are mean of n = 4–5 ± standard error of the mean. No statistical analysis was done due to the limited number of animals.

Therefore, our optimization goal was to establish a structure–activity relationship (SAR) in which the PK properties are optimized for once-daily dosing and the peak-to-trough ratio is very low to avoid acute, C_{max}-driven hypotension but maintain a safe and long-lasting reduction of BP.

The kinetic profile could be optimized and translated into a pronounced, long-lasting and dose-dependent BP reduction in conscious hypertensive rats. These data indicated that BAY-747 might be optimal with respect to the kinetic profile and highly effective as a BP-lowering drug.

A potential limitation of drugs acting on the NO–cGMP pathway is the development of tolerance and tachyphylaxis, which is well described for organic nitrates and NO donors. Therefore, we conducted a longer-term treatment study in hypertensive rats by daily administration of BAY-747 over 12 days. We did not observe a decrease in the BP-lowering effect over time or any hints of tachyphylaxis. This is also in line with previous findings with riociguat and vericiguat in which no tachyphylaxis was observed ex vivo in blood vessels (Follmann et al., 2017). Even in the clinic, long-term administration of riociguat and vericiguat did not show tachyphylaxis. Recently, it was reported that consistent BP reductions were observed in praliciguat-treated healthy volunteers over a period of 21 days (Hanrahan et al., 2019).

Because it is known that a significant proportion of HT patients do not or do not adequately respond to hypertensive treatment, BAY-747 was also tested in models that reflect treatment-resistant HT. Hypertensive rats were treated with a dose-adjusted combination of antihypertensive drugs to maximize their BP-lowering efficacy. Notably, BAY-747 was still able to elicit additional BP lowering on top of these combinations. Thus, the sGC stimulator BAY-747 could be useful for patients with resistant and maybe even refractory HT, which is defined as uncontrolled BP despite use of ≥5 antihypertensive agents of different classes (Acelajado et al., 2019).

The efficacy on top of a combination of antihypertensives could be related to the alternative mode of action of sGC stimulators that act via a different signalling cascade compared to the approved and guideline-recommended antihypertensive drugs and combinations thereof. The high efficacy of BAY-747 could mechanistically be directly related to the active stimulation of cGMP production in vascular smooth muscle cells. This mode of action is also supported by the concentration-dependent vasodilation of ex vivo blood vessels in our experiments. In addition, BAY-747 also stimulates sGC and cGMP production in the absence of NO. This could also be important for the use in HT, because vasodilation by endogenous NO could be impaired in these patients due to endothelial dysfunction. To provide further evidence that BAY-747 acts independently of NO *in vivo*, the BP-lowering action of BAY-747 was tested in L-NAME-treated hypertensive (transgenic (mREN2)27) rats. Despite chronic blockage of NO production with L-NAME over 7 days, the sGC stimulator BAY-747 was able to significantly reduce BP (Figure 9).

However, to further substantiate and broaden the findings, BAY-747 was also tested in a newly established hypertensive dog model combining unilateral renal wrapping and renal artery stenosis. These renal wrap dogs develop HT and do not adequately respond to antihypertensive drugs (Vogel et al., 2021). This dog model reflects stable but also therapy-resistant HT. It was previously shown that the sGC stimulator BAY 41-2272, in contrast to Standard of Care (SoC) antihypertensives, caused a dose-dependent and stable BP reduction in this model, when given alone or in combination with antihypertensive therapy. In fact, BAY-747 also significantly reduced BP in these hypertensive dogs, suggesting an efficacy of BAY-747 in rHT. These data demonstrate that sGC stimulators might overcome the limitations of currently used antihypertensives and might offer a powerful treatment alternative for rHT patients.

In hypertensive rats and dogs, BAY-747 leads to a compensatory HR increase after acute administration, as seen with many other vasodilating drugs, including other sGC stimulators (Follmann et al., 2017). This reflex tachycardia could become a limitation for the use of BAY-747 and other sGC stimulators as antihypertensives, especially in patients with cardiac co-morbidities and heart failure. Interestingly, upon repeated administration of BAY-747 in SH rats, the transient moderate HR increase was observed only at the first 3–4 days. This initial tachycardia completely disappeared during the treatment period. In addition, an HR increase was not seen in the triple combination experiment of BAY-747 in combination with the beta-blocker atenolol, although the BP-reducing effect of the BAY-747 plus atenolol combination was significantly higher compared to the stand-alone treatments.

An additional interesting finding is the fact that the sGC stimulator BAY-747 is able to penetrate into the brain. Therefore, this molecule might also have beneficial effects in neurodegenerative diseases and dementias. It was shown recently that BAY-747 enhances memory function *in vivo* in a rat model of memory acquisition (Nelissen et al., 2022). The exact mode of action is not fully understood, but the vascular mode of action could contribute to the overall efficacy of BAY-747 on learning and memory.

In summary, the results from PD *in vivo* profiling of BAY-747 in rats and dogs in models with malignant HT and rHT were used in PK/PD models to predict a minimal effective concentration in humans (leading to a minimal significant CV effect). Based on that, the human predicted drug concentration–time (c/t) profiles for once-daily administration at steady state (using rat or dog PK data) lead to a very low to moderate peak-to-trough ratio and a low efficacious dose, which would be optimal human PK parameters for such an indication.

The he novel sGC stimulator BAY-747, representing a new class of sGC stimulators, might provide an effective therapy option for malignant, therapy-resistant and even refractory HT and might have potential in neurodegenerative diseases and vascular dementia.

AUTHOR CONTRIBUTIONS

Frank Wunder: Conceptualization (equal); investigation (equal); project administration (equal); visualization (lead); writing—original draft (lead); writing—review and editing (equal). **Johannes-Peter Stasch:** Conceptualization (equal); data curation (equal); investigation (equal); writing—original draft (supporting). **Andreas Knorr:** Conceptualization (equal); investigation (equal); visualization (supporting); writing—review and editing (supporting). **Thomas Mondritzki:** Conceptualization (equal); data curation (equal); formal analysis (equal); investigation

(equal); visualization (equal); writing—original draft (supporting); writing—review and editing (equal). **Damian Brockschnieder:** Investigation (equal); methodology (supporting); writing—original draft (supporting). **Eva-Maria Becker-Pelster:** Investigation (equal); writing—original draft (supporting); writing—review and editing (supporting). **Peter Sandner:** Conceptualization (equal); data curation (supporting); investigation (equal); writing—original draft (supporting); writing—review and editing (equal). **Hanna Tinel:** Data curation (equal); investigation (equal); visualization (supporting); writing—original draft (supporting); writing—review and editing (supporting). **Gorden Redlich:** Data curation (equal); formal analysis (equal); investigation (equal); visualization (equal); writing—original draft (supporting); writing—review and editing (supporting). **Ingo V. Hartung:** Conceptualization (equal); investigation (equal); project administration (equal); resources (equal); writing—original draft (supporting); writing—review and editing (equal). **Alexandros Vakalopoulos:** Conceptualization (equal); project administration (equal); resources (equal); visualization (supporting); writing—original draft (supporting); writing—review and editing (supporting). **Markus Follmann:** Conceptualization (equal); project administration (equal); resources (equal); writing—original draft (equal); writing—review and editing (supporting).

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CONFLICT OF INTEREST STATEMENT

All authors are or were, at the time of the studies, employees of Bayer AG. J-PS is a senior advisor of Bayer AG. FW, J-PS, AK, E-MB-P, GR, IVH, AV and MF have a patent WO2014068099(A1) issued. The other authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data are available on request from the authors.

DECLARATION OF TRANSPARENCY AND SCIENTIFIC RIGOUR

This Declaration acknowledges that this paper adheres to the principles for transparent reporting and scientific rigour of preclinical research as stated in the *BJP* guidelines for Design & Analysis and Animal Experimentation and as recommended by funding agencies, publishers and other organizations engaged with supporting research.

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