Supporting information

Material and methods

Pharmacokinetic analysis

Rat plasma concentrations of Lu AF41228 and Lu AF58027 were determined using Ultra performance liquid chromatography (UPLC) coupled to tandem mass spectrometry (MS/MS). Samples from animals dosed with Lu AF41228 were precipitated with acetonitrile containing internal standard and 0.1% ammonium hydroxide. After centrifugation and dilution, 10 µl of the supernatant was injected onto the chromatographic system (Waters Acquity, Milford, U.S.A). The mobile phase consisting of 0.1% ammonium hydroxide in methanol was pumped through the column (Acquity UPLC Phenyl BEH 30x2.1mm, 1.7µm) using a gradient with a flow rate of 0.6 µl/min. Samples containing Lu AF58027 were prepared under acidic conditions, precipitated with acetonitrile containing internal standard and or 0.1% formic acid. Lu AF5027 was eluted through the column (Acquity UPLC C18 SB HSS 30x2.1mm, 1.8 µm) with a mobile phase consisting of 0.1% formic acid in methanol. MS/MS detection was performed with an Applied Biosystems Sciex API 4000 instrument in positive-ion electrospray ionisation mode (Sciex 4000, AB Sciex, Foster City, USA). Lu AF41228 and Lu AF58027 were detected at a parent > daughter mass to charge ratio (m/z) of 276.24 > 206.10 and 487.31 > 143.20. Nitrogen was used for the nebulizer and collision gases. The retention times for Lu AF41228 and LU58027 was 0.93 and 1.02 min., respectively. The peak area correlated linearly with the plasma and brain concentration of the analytes in the range of 1.00 – 1000 ng/ml plasma. Data acquisition and analysis was performed using Analyst software, version 6.1 (AB Sciex, Foster City, CA). During data evaluation, measured plasma concentrations were converted to unbound concentrations by multiplying the total concentration with the free fractions of Lu AF41228 (28%) and Lu AF58027 (6%) in rat plasma measured in vitro by standard equilibrium dialysis.

Guinea pig isolated heart studies

Male guinea pigs (Dunkin Hartley Crl:HA, Charles River, DE), weighing 300-700g were anaesthetized with an intraperitoneal injection of Na-Pentothal to effect (<150mg/kg) combined with Heparin (0.2ml/kg, 5000units/ml) and immobilized on an operating table. Following tracheotomy, the guinea pig was ventilated with air (tidal volume 3 ml per stroke, 70 strokes per min) and a cannula was placed in the ascending aorta and sutured. In situ perfusion was initiated immediately using a modified Krebs-Henseleit solution. The perfusion fluid was composed of the following (in mM): NaCl 118.1, KCl 4.7, CaCl2-2H2O 2.5, KH2PO4 1.2, MgSO4-7H2O 1.6, CH3COCO2Na 2.0, NaHCO3 24.9, C6H12O6-H2O (D-glucose monohydrate) 5.6 (5% CO2, 95% O2, 36.5 degrees C, pH 7.4). The whole heart was mounted in a IH-SR isolated heart apparatus (Hugo Sachs, DE) and perfused according to the Langendorff principle (Bell *et al.*, 2011). Hearts were spontaneously beating at sinus frequency. All signals were amplified using PlugSys amplifiers (Hugo Sachs) and data was recorded using LabChart7 (ADIstruments, UK). Via the left atrium a small balloon was placed in the left ventricle to measure the left ventricular pressure and Ag-AgCl electrodes were placed on the heart to record the ECG. Compound or vehicle was added to the buffer solution administered at a constant perfusion pressure of 60 mmHg. DMSO concentrations were kept at 0.1% during all experiments.

Results

Expression of PDE1

Supplementary Table S1. Primers used for detection of phosphodiesterase type 1 subtypes.

Gene	Sense	Antisense	Expected size
Pde1a	5´aatgatcttatcaaccgcttcaag´3	5´cgagtctgtcaggagagaaaacg´3	747 bp
Pde1b	5´attcccacagtgtttctgatgag´3	5´ggctgacttttagacttggaatc´3	779 bp
Pde1c	5´gaagacatccttcaggttacgg´3	5´aagtggtggttctccagtacag´ 3	792 bp

Studies in isolated vascular segments

Supplementary Table S2. Potency and maximal relaxation of phosphodiesterase inhibitors in mesenteric and femoral small arteries.

	Mesenteric U46619		Femoral U46619		Mesenteric Noradrenaline		Femoral Noradrenaline	
	pD_2	Max relax %	pD_2	Max relax %	pD_2	Max relax %	pD_2	Max relax %
Sildenafil	9.02±0.11	94±2	7.88±0.23	71±4	7.29±0.14	79±4	7.22±0.24	71±6
Milrinone	7.17±0.43 *	76±8	7.15±0.31	58±6	6.41±0.17 *	76±6	6.45±0.33	65±10
Lu AF58207	7.22±0.20 *	90±6	6.11±0.21 *	65±8	6.47±0.07 *	101±4	6.26±0.10 *	96±6 *
Lu AF41228	7.01±0.19 *	84±6	5.86±0.52 *	64±24	6.21±0.12 *	64±5	6.22±0.30 *	59±10

The results are means ± s.e.m. of 5-7 animals. pD_2=-logEC_{50}, where EC_{50} is the concentration inducing a halfmaximal response. Max relax is the maximum relaxation expressed relative to the tone evoked by the thromboxane analogue, U46619 or noradrenaline. * $P<0.05$, parameter significantly different from those for sildenafil relaxation curves, One–way ANOVA followed by t-test.

Supplementary Table S3. Contraction level induced by (A) phenylephrine and (B) U46619 and potency and maximal relaxation of phosphodiesterase type 1inhibitors in mesenteric small arteries in the absence and the presence of an inhibitor of guanylate cyclase, ODQ, an inhibitor of adenylate cyclase, SQ22536 or the combination.

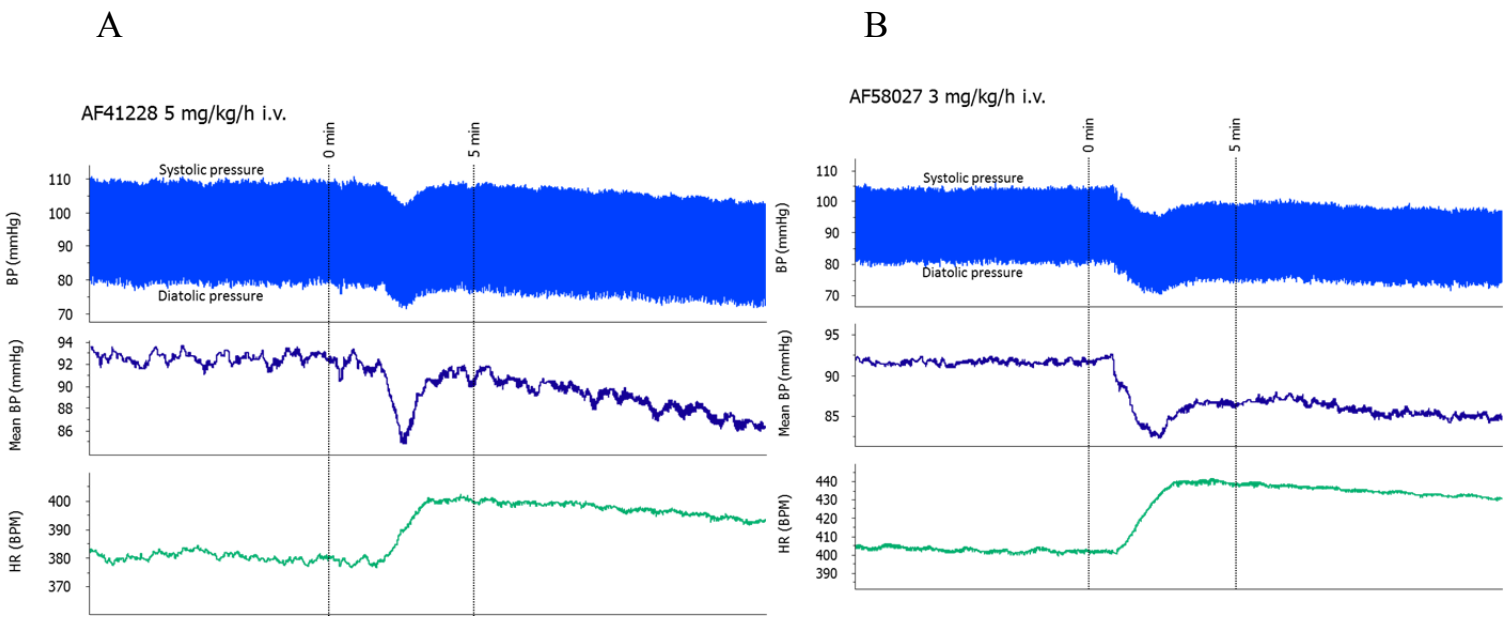
A	**N**	**PhE**	**Lu AF41228**		**N**	**PhE**	**Lu AF58027**	
		Tension Nm^{-1}	pD_2	Max relax %		Tension Nm^{-1}	pD_2	Max relax %
Vehicle	6	4.2±0.4	6.81±0.09	95.3±0.9	6	3.3±0.5	7.44±0.11	98.5±0.2
ODQ	6	5.1±0.4	5.68±0.19 *	71.3±6.2 *	7	3.5±0.3	6.07±0.05 *	95.3±0.7
SQ22.536	6	3.7±0.4	5.21±0.08 *	62.4±8.6 *	5	2.9±0.3	6.85±0.11 *	96.3±0.9
ODQ plus SQ22.536	5	3.8±0.4	4.87±0.20 *	51.8±10.3 *	6	3.5±0.6	6.02±0.03 *	95.0±0.6

B	N	U46619	Lu AF41228		N	U46619	Lu AF58027	
		Tension Nm^{-1}	pD_2	Max relax %		Tension Nm^{-1}	pD_2	Max relax %
Vehicle	6	3.5±0.5	6.27±0.09	94.3±1.4	6	2.5±0.3	7.44±0.11	96.1±1.7
ODQ	6	5.5±0.7	5.25±0.07 *	63.8±12.4 *	7	3.1±0.2	6.26±0.06 *	93.2±0.9
SQ22.536	6	2.8±0.6	5.64±0.05 *	74.0±4.0 *	5	3.0±0.4	6.45±0.07 *	90.0±1.6
ODQ plus SQ22.536	5	4.2±0.2	ND	18.7±3.5 *	6	3.8±0.3	5.89±0.04 *	91.7±2.0

The results are means ± s.e.m. of segnments from a number (N) of animals. pD_2=-logEC_{50}, where EC_{50} is the concentration inducing a halfmaximal response. Max relax is the maximum relaxation expressed relative to the tone evoked by the thromboxane analogue, U46619 or phenylephrine (PhE). ND=not determined as maximum relaxation is less than 50%.

Hemodynamic studies

In anesthetized rats, infusion of Lu AF41228 and Lu AF 58027 reduced systolic and diastolic pressure with a reduction in mean arterial blood pressure peaking approximately 150 s after start of infusion followed by increase in heart rate peaking 40-50 s later followed by a more slow decrease in mean arterial blood pressure (Supplementary Fig 1A and B).

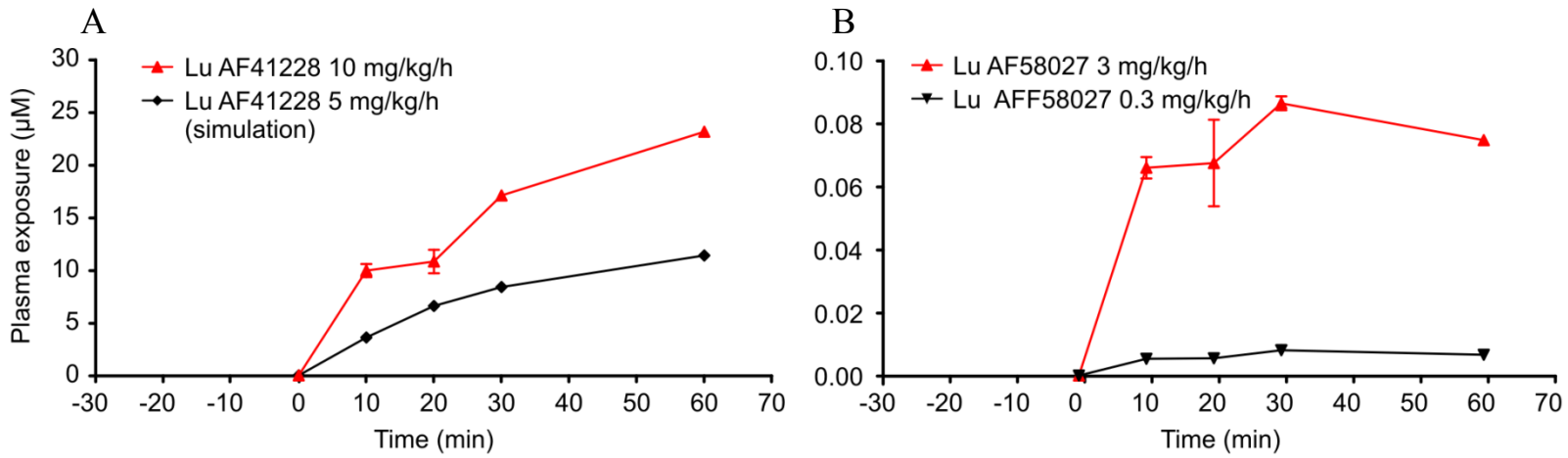


Supplementary Figure S1. Original recordings showing the effect on systolic/diastolic blood pressure (BP), mean arterial BP, and heart rate after infusion of respectively, (A) Lu AF41228 and (B) Lu AF58027 in anesthetized rats.

Pharmacokinetic studies

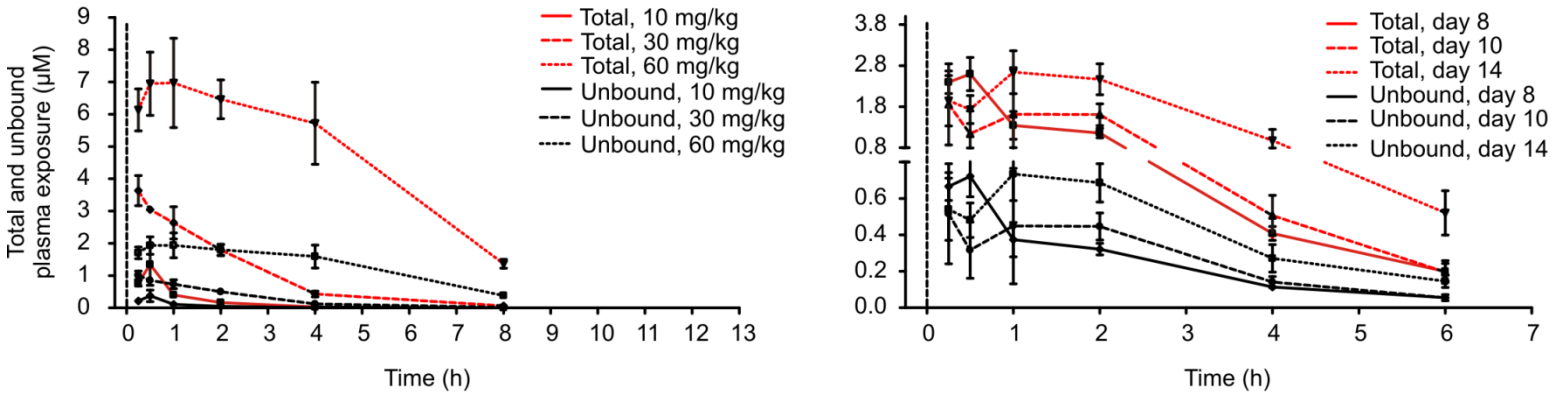
In the anesthetized rat studies, after total and unbound plasma exposure to Lu AF41228 and Lu AF58027 after intravenous administration was measured in satellite animals in parallel to the hemodynamic measurements (Supplementary Fig. 1A, B). The total and unbound plasma exposure to Lu AF41228 after administration of different doses of Lu AF41228 (Supplementary Fig. 2A) or

twice daily administration of 30 mg/kg of Lu AF41228 for 7 days (Supplementary Fig. 2B) was also measured in parallel to the hemodynamic measurements in rats in the telemetry study.



Supplementary Figure S2. Plasma exposure to Lu AF41228 and Lu AF58027 after intravenous administration in anesthetized rats. (A) Unbound plasma exposure of Lu AF41228 from satellite animals (n=2). The unbound plasma exposure was calculated from the total plasma exposure and a plasma protein binding of 72.2%. For the 5 mg/kg/h dose, plasma exposure is presented as an i.v. PK simulation since plasma exposure was not recorded at this dose. (B) Plasma exposure of Lu AF58027 from satellite animals (n=2 per dose). The unbound plasma exposure was calculated from the total plasma exposure and a plasma protein binding of 93.9%.

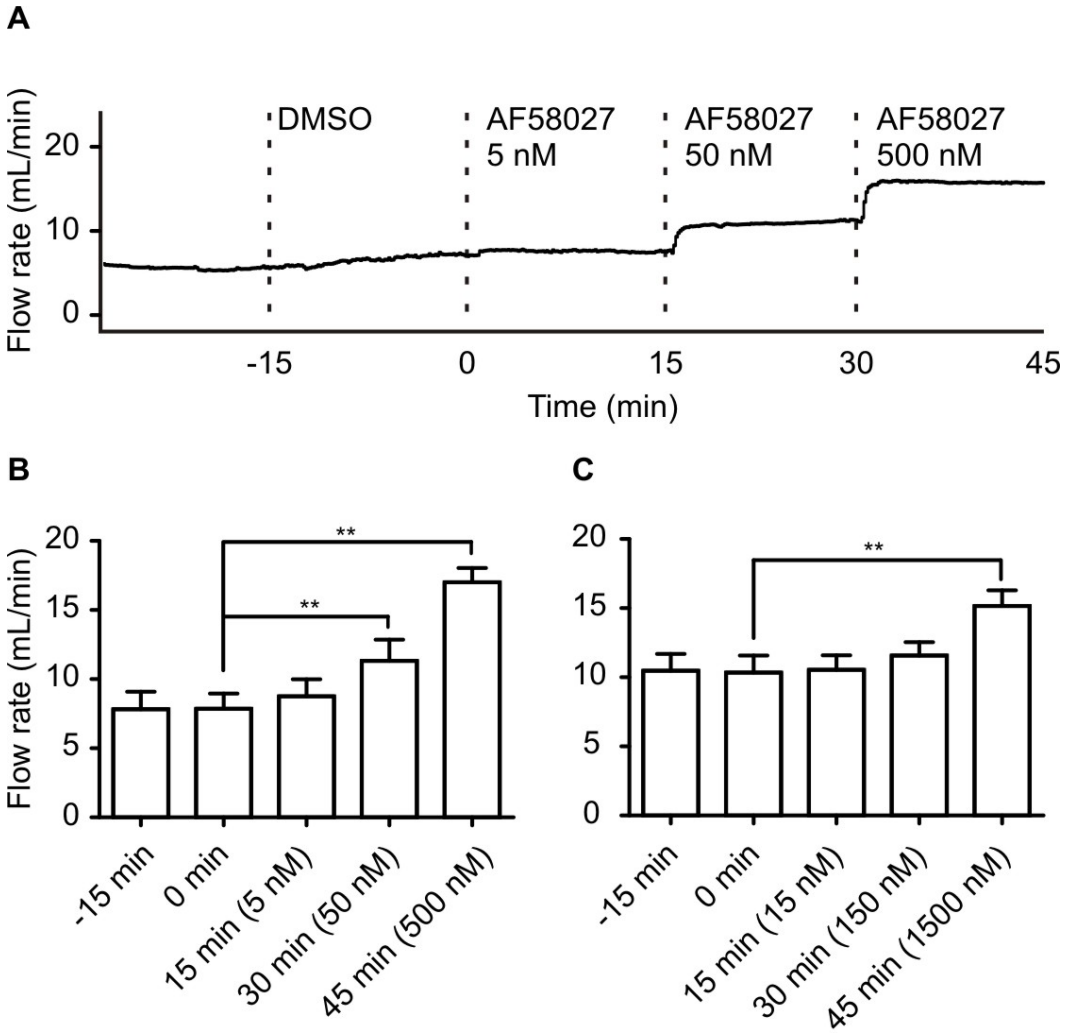
A **B**



Supplementary Fig. S3. Plasma exposure to Lu AF41228 after oral administration in telemetered rats. (A) Exposure in satellite animals after dosing with Lu AF41228 10, 30 and 60 mg/kg (n=3). The unbound plasma exposure was calculated from the total plasma exposure and a plasma protein binding of 72.2%. (B) Exposure in satellite animals after dosing with Lu AF41228 30 mg/kg twice daily for 7 days (n=3). The unbound plasma exposure was calculated from the total plasma exposure and a plasma protein binding of 72.2%.

Guinea pig isolated heart studies

Following evaluation of enzymatic activity and effects on isolated blood vessels, Lu AF41228 and Lu AF58027 were evaluated in spontaneously beating Langendorff isolated guinea pig hearts using a range of PDE1-selective concentrations. Here, Lu AF41228 (15, 150 and 1500 nM) and Lu AF58027 (5, 50 and 500 nM) had no effects on the ECG (PR, QRS and QT intervals), cardiac contraction (left ventricular pressure and ΔLVP/Δt max) or heart rate. However, both compounds markedly increased the perfusion rate recorded under constant pressure, thus signifying a decrease in vascular resistance (Supplementary Fig. 3). AF41228 resulted in an average increase in coronary flow rate from 10.3 to 15.2 mL/min (152±14%) at 1500 nM and AF58027 resulted in an average increase in flow rate from 7.8 to 11.3 mL/min (146±10%) at 50 nM and from 7.8 to 17.0 mL/min (233±30%) at 500 nM.



Supplementary Fig. S4. Coronary flow increases in response to PDE1 inhibition in isolated guinea pig heart. After equilibrium was reached, baseline was recorded for 15 minutes and hearts were then perfused with vehicle solution for 15 minutes followed by consecutive infusions of compound at 15 minute intervals. (A) Example of dose-dependent increase in coronary flow rate after infusion of increasing concentrations of Lu AF58027 at 15-min intervals. (B) Dose-dependent increase in coronary flow in response to

cumulative addition of Lu AF58027 (white bars). Response reached statistical significance at the two highest doses (50 and 500 nM; $p<0.01$, n=6). (C) Dose-dependent increase in coronary flow in response to cumulative addition of Lu AF41228 (white bars). Response reached statistical significance at the highest dose (1500 nM; $p<0.01$, n=6).