Supplementary Materials

A simple strategy for low-cost portable monitoring of plasma drug concentrations using a sustainable boron-doped-diamond chip

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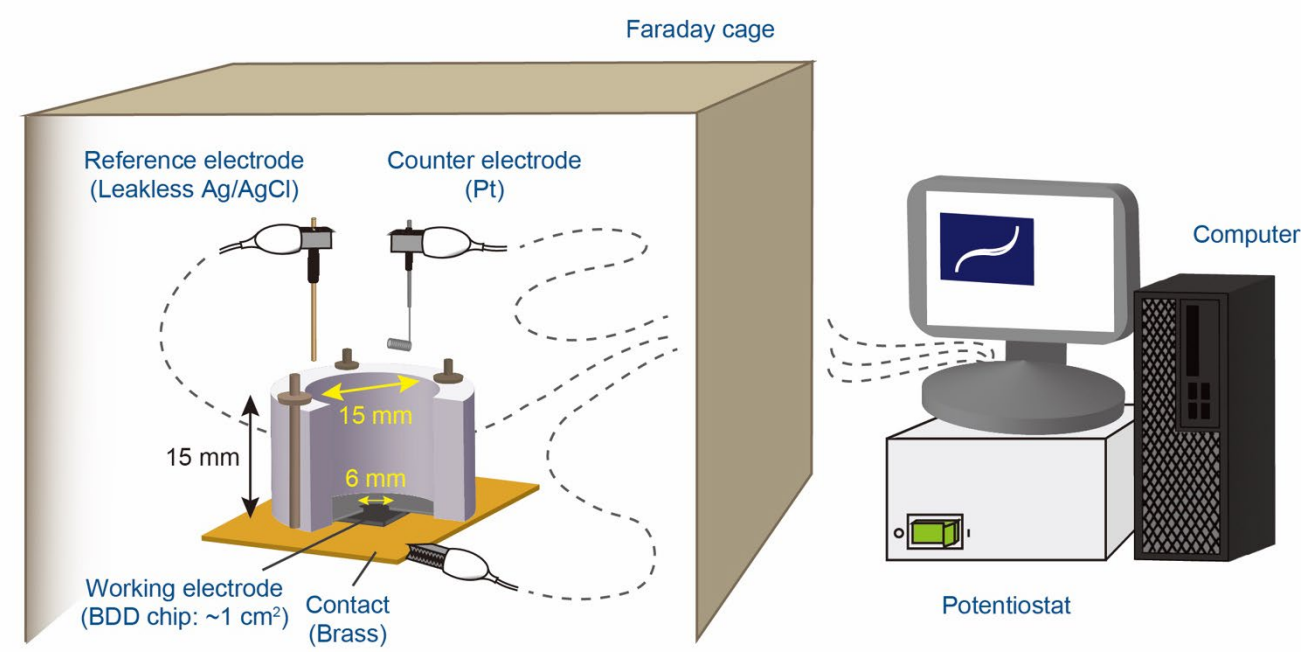
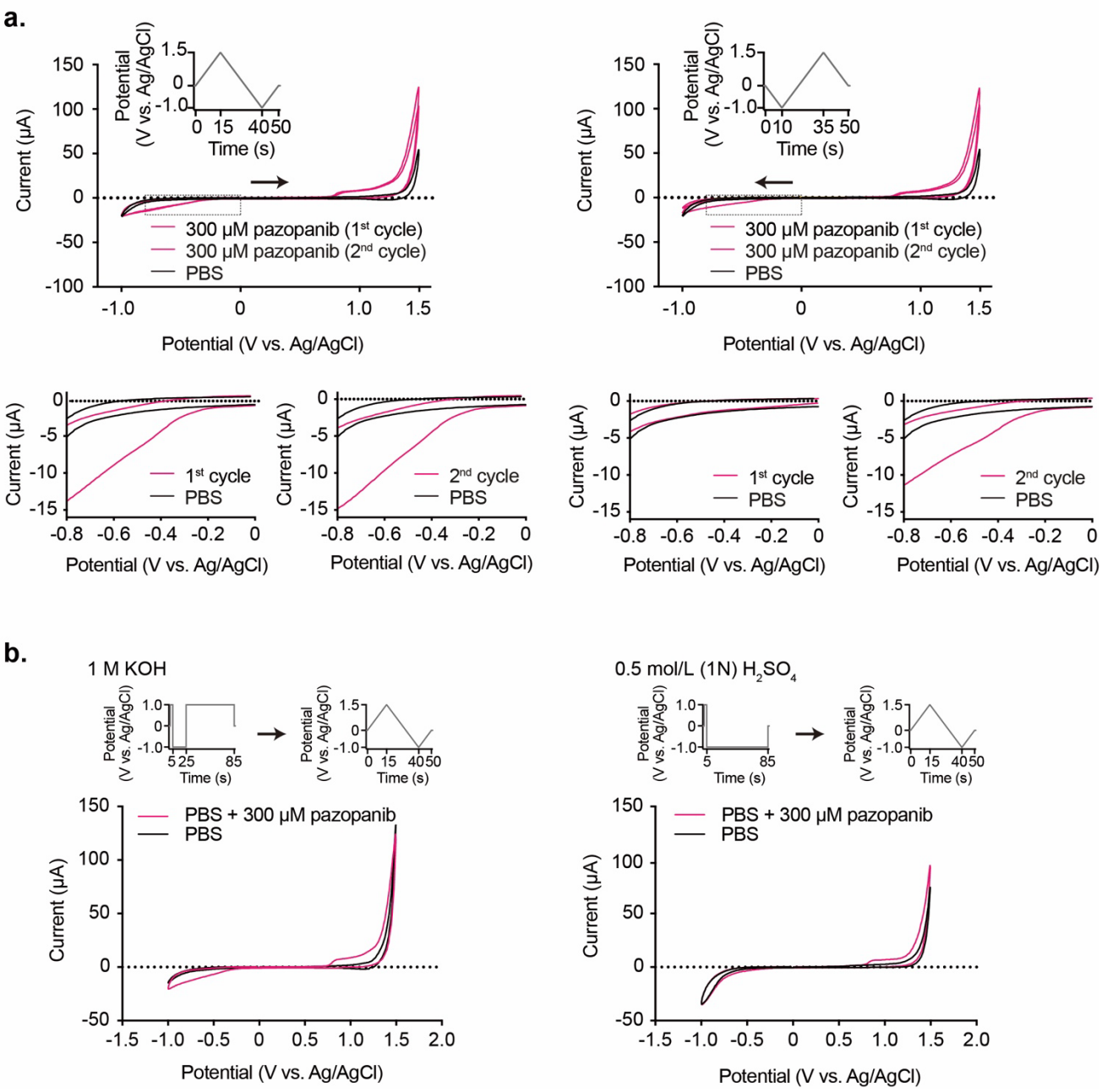
Supplementary Figures



Supplementary Figure 1 The tabletop system for electrochemical drug monitoring.

In this experimental setup, a square-shaped plate chip of 1% boron-doped diamond (BDD; ~1 cm^2) served as a working electrode and was overlaid with a small cylindrical chamber (internal diameter: 15 mm, external diameter: 21 mm, height: 15 mm, and volume: 2.3 mL), which has a hole 6 mm in diameter at the bottom. Under the BDD chip, a brass plate was placed and connected to a potentiostat regulated by a desktop computer. The chamber housed a reference electrode (Ag/AgCl) and a counter electrode (platinum: Pt). A hundred and twenty microlitres of a control or analyte-containing solution was placed in the chamber for electrochemical detection of the analyte.



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48

49 **Supplementary Figure 2 The potential protocol for electrochemical detection of**

50 **pazopanib.**

51 ***a***, Effects of different redox states of pazopanib on the electrochemical reaction. Pazopanib at

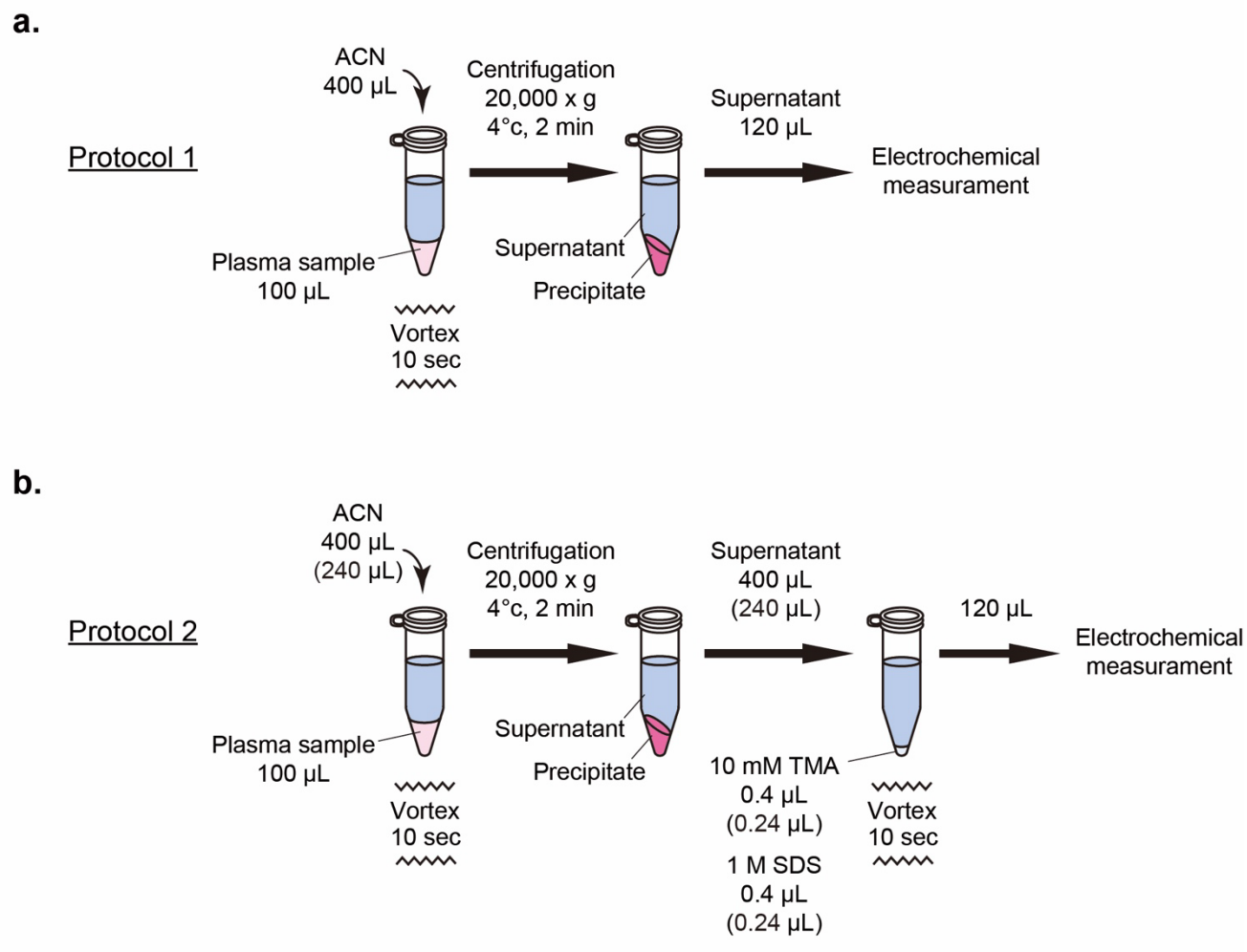
52 300 μM dissolved in phosphate-buffered saline (PBS) was analysed on a boron-doped diamond (BDD)

53 chip (chip ID: B) in the tabletop system by cyclic voltammetry via two different potential protocols as

follows. In *left panels*, the applied potential was scanned in the positive direction (*arrow*) from 0 to 1.5 V (versus Ag/AgCl) to first oxidise pazopanib and then the potential was shifted in the negative direction to –1.0 V at a sweep rate of 0.1 V s^{-1}. In *right panels*, the potential was scanned in the negative direction (*arrow*) from 0 to –1.0 V (versus Ag/AgCl) to reduce the compound and then shifted in the positive direction to 1.5 V at the same sweep rate. These protocols are shown in the *insets*. The cyclic voltammetry analysis of pazopanib was carried out twice (*magenta curves*). For control data, PBS alone was tested (*black curves*). The traces marked by *doted boxes* in *upper panels* are enlarged in *lower panels*. The results indicate that the reaction of pazopanib reduction requires the oxidised form of the compound.

b, Effects of different surface conditions of a BDD chip (chip ID: B) on pazopanib detection. In the *left panel*, the chip was bathed in 1 M KOH and underwent the following protocol. After being elevated abruptly from 0 to 1.0 V (versus Ag/AgCl), the applied potential was kept at 1.0 V for 5 s, stepped to –1.0 V for 20 s, clamped at 1.0 V for 60 s, and then returned to 0 V. This pre-treatment results in oxygen (O)-terminated electrode surface.[1] After that, the electrode was washed with ultrapure water and subjected to cyclic voltammetry analysis of PBS alone (*black curve*) or PBS containing pazopanib (300 μM) (*magenta curve*) by the protocol used in *left panels* of ***a*** (sweep rate: 0.1 V s^{-1}, potential window: −1.0 to 1.5 V, initial potential: 0 V versus Ag/AgCl). In the *right panel*, first, the electrode surface was hydrogen (H)-terminated in a solution containing 0.5 M H_2SO_4 in a pre-treatment where the potential was kept at 1.0 V for 5 s (initial potential: 0 V) and clamped at –1.0 V for 80 s.

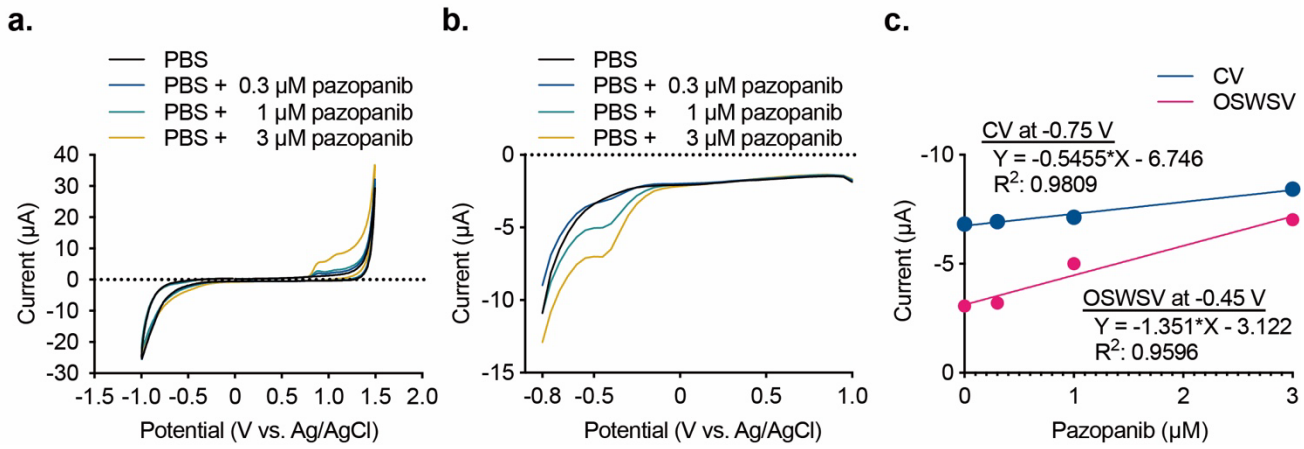
After the electrode was washed with water, cyclic voltammetry analysis was performed on either PBS alone or PBS containing pazopanib (300 μM). For potential protocols, see *insets* in the two *panels*. Note that the cathodic current induced by the drug at negative potentials was detected on the O-terminated surface (*left panel)* but not on the H-terminated surface (*right panel*).



Supplementary Figure 3 Sample preparation procedures for electrochemical measurements.

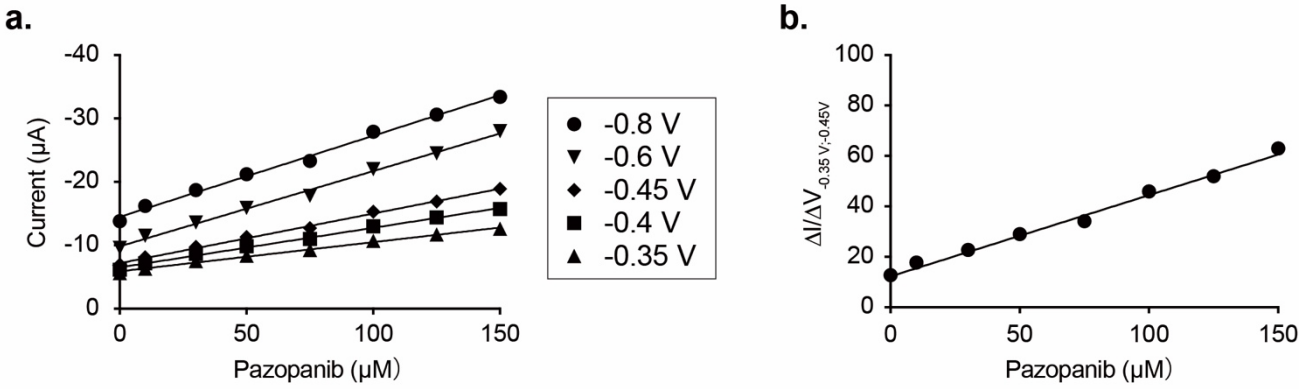
Rat and patient plasma samples were processed for electrochemical measurement as displayed in the *panels*. In 'Protocol 1' (*panel* ***a***), a rat sample was mixed with 400 µL of acetonitrile (ACN) by vortexing for 10 s and centrifuged at 20,000 × *g* for 2 min (4 °C). Next, 120 µL of the supernatant was subjected to the electrochemical assay (**Figure 2b**). In 'Protocol 2' (*panel* ***b***), after a rat or clinical sample was treated with ACN, 400 µL of the supernatant was obtained and mixed by vortexing for 10 s with a 10 mM tetramethylammonium (TMA) solution and a 1 M sodium dodecyl sulphate (SDS)

87 solution (0.4 μL for each). Finally, 120 μL of this sample was subjected to electrochemical
88 measurement. This method was used for the assays in **Figures 3a−c**, **5**, and **6** and **Supplementary**
89 **Figures 5**, **8**, **9**, and **10**. For analysis of whole blood of the rats orally given pazopanib (**Figure 4** and
90 **Supplementary Figures 6** and **7**), the volume of the plasma samples and solutions was scaled to three-
91 fifths, as specified in parentheses.



Supplementary Figure 4 **Detection of pazopanib by different voltammetry procedures.**

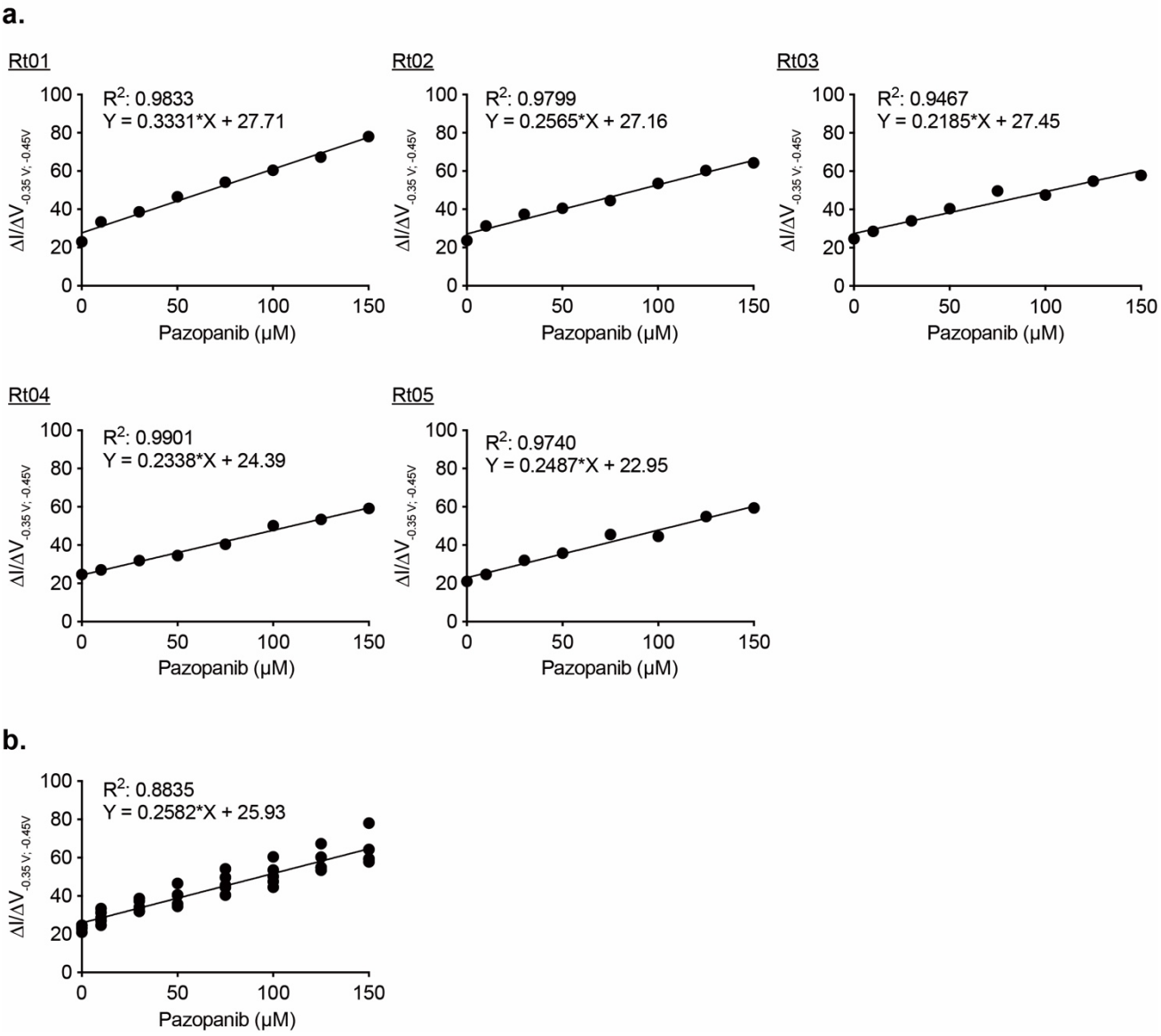
Phosphate-buffered saline (PBS) alone or PBS containing pazopanib at different concentrations (0.3, 1.0, or 3.0 µM) was electrochemically analysed on a boron-doped diamond (BDD) chip (chip ID: G) in the tabletop system with two different methods: cyclic voltammetry (CV) in *panel **a*** (sweep rate: 0.1 V s^{-1}, potential window: –1.0 to 1.5 V, initial potential: 0 V versus Ag/AgCl) and Osteryoung square wave stripping voltammetry (OSWSV) in *panel **b*** (deposition potential: 1.4 V vs Ag/AgCl, deposition time: 30 s, potential range: –0.8 to 1 V, ΔE: 50 mV, square-wave frequency: 10 Hz, pulse amplitude: 50 mV). The current amplitudes at –0.75 V in the cyclic voltammogram (***a***) and those at –0.45 V in the OSWS voltammogram (***b***) are plotted in *panel **c*** as *blue* and *magenta curves*, respectively, as a function of the drug concentrations. The slope and R^2 values of the regression lines are indicated.



c.

Sampling data	Slope value	Equation	R^2 value
Current at -0.8 V	-0.1279	Y = -0.1279*X - 14.50	0.9942
Current at -0.6 V	-0.1187	Y = -0.1187*X - 9.855	0.9951
Current at -0.45 V	-0.07823	Y = -0.07823*X - 7.206	0.9967
Current at -0.4 V	-0.06254	Y = -0.06254*X - 6.526	0.9955
Current at -0.35 V	-0.04602	Y = -0.04602*X - 5.911	0.9943
$\Delta I/\Delta V_{-0.35\ V;-0.45\ V}$	0.3221	Y = 0.3221*X + 12.95	0.9926

Supplementary Figure 5 Optimisation of the protocol for calibration curve construction. From the voltammogram depicted in **Figure 3b** (BDD chip ID: D), the current amplitudes detected at –0.35, –0.4, –0.45, –0.6, and –0.8 V in the measurements of different pazopanib concentrations (0−150 μM) were extracted and are plotted in *panel **a*** to show the calibration curves. For comparison, the calibration curve from **Figure 3c**, which was obtained from $\Delta I/\Delta V_{-0.35\ V;-0.45\ V}$ values in the voltammogram of **Figure 3b**, is displayed in *panel **b***. The slope, the equation for the linear regression line fitting the data points, and R^2 in each analysis are listed in *panel **c***.

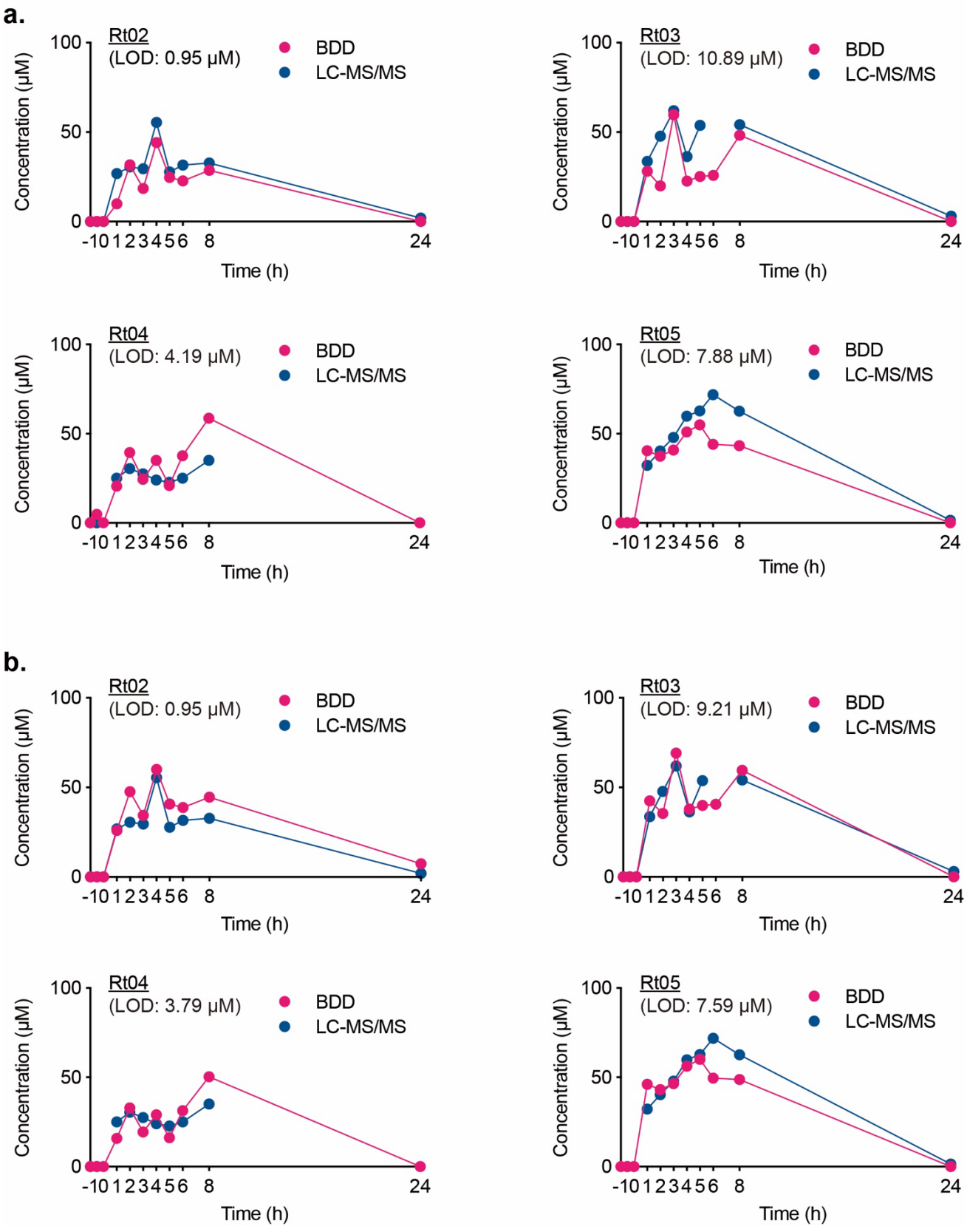


Supplementary Figure 6 Calibration curves for measurements of pazopanib concentrations in the plasma of systemically treated rats.

a, Calibration curves for individual rats. From the five rats assayed in **Figure 4**, 900 μL of whole blood was collected immediately before oral administration of pazopanib (see the *main text* and **Methods**). The extracted plasma was spiked with the drug at different concentrations. These samples (0 to 150 μM pazopanib; see **Figure 3b** and **c**) were electrochemically analysed using a boron-doped

diamond (BDD) chip in the tabletop system (chip ID: F) by Osteryoung square wave stripping (OSWS) voltammetry (for the potential protocol, see **Figure 3a**). The obtained $\Delta I/\Delta V_{-0.35\ V;-0.45\ V}$ values are plotted against the drug concentrations in the *panels*. Animal ID numbers and slope and R^2 values of the regression line are presented in each *panel*. These calibration curves and slope values are used in **Figure 4a–d** and **Figure 6**, respectively.

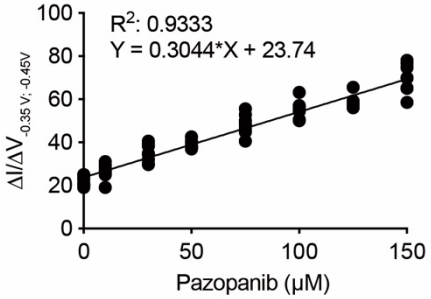
b, The 'general' calibration curve. In the *panel*, all the data on the five animals (*panel **a***) are plotted as a function of the drug concentrations and are fitted to a regression line, whose slope and R^2 values are shown. This calibration curve is used in **Figure 4e–h**.


a.
Rt02
(LOD: 0.95 μM)
BDD
LC-MS/MS
Concentration (μM)
Time (h)
Rt03
(LOD: 10.89 μM)
Rt04
(LOD: 4.19 μM)
Rt05
(LOD: 7.88 μM)
b.
Rt02
(LOD: 0.95 μM)
Rt03
(LOD: 9.21 μM)
Rt04
(LOD: 3.79 μM)
Rt05
(LOD: 7.59 μM)

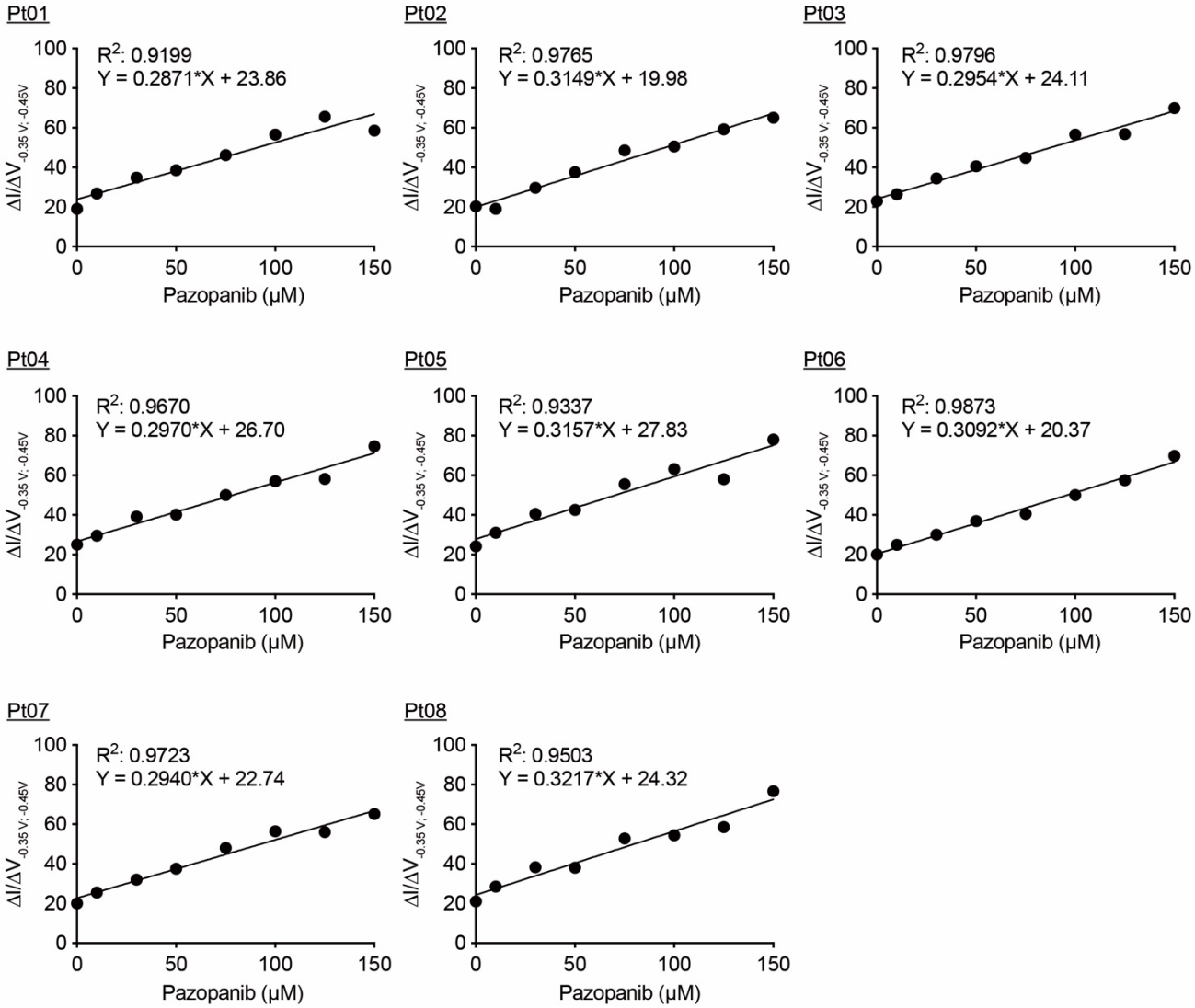
Supplementary Figure 7 Individual measurements of pazopanib in the plasma of the systemically treated rats.

Shown in *panel* ***a*** are the individual data from longitudinal measurements of plasma pazopanib concentrations by the tabletop system based on a boron-doped diamond (BDD) chip (chip ID: F) and by liquid chromatography–tandem mass spectrometry (LC–MS/MS) for four healthy rats (animal IDs: Rt02−05). The experimental procedure for the electrochemical measurements was basically the same as the one used in **Figure 4a**; for each rat, to convert $\Delta I/\Delta V_{-0.35\ V;-0.45\ V}$ values to analyte concentrations, an individual calibration curve was employed (**Supplementary Figure 6a** and 'Method #1" in the *main text*). In *panel* ***b***, the conversion was carried out via the 'general' calibration curve, which was obtained in **Supplementary Figure 6b** from the data on the five rats [animal IDs: Rt01 (**Figure 4a**) and Rt02−05] ('Method #2" in the *main text*). Of note, as for LC–MS/MS measurements, the data at 6 hr for Rt03, those immediately before the drug administration and at 24 hr for Rt04, and those immediately and 1 hr before the drug administration for Rt05 were missing due to collection of an insufficient volume of blood (our technical error; see **Methods**). Limits of detection (LODs) obtained in the electrochemical assays are indicated in the *panels*.

a.

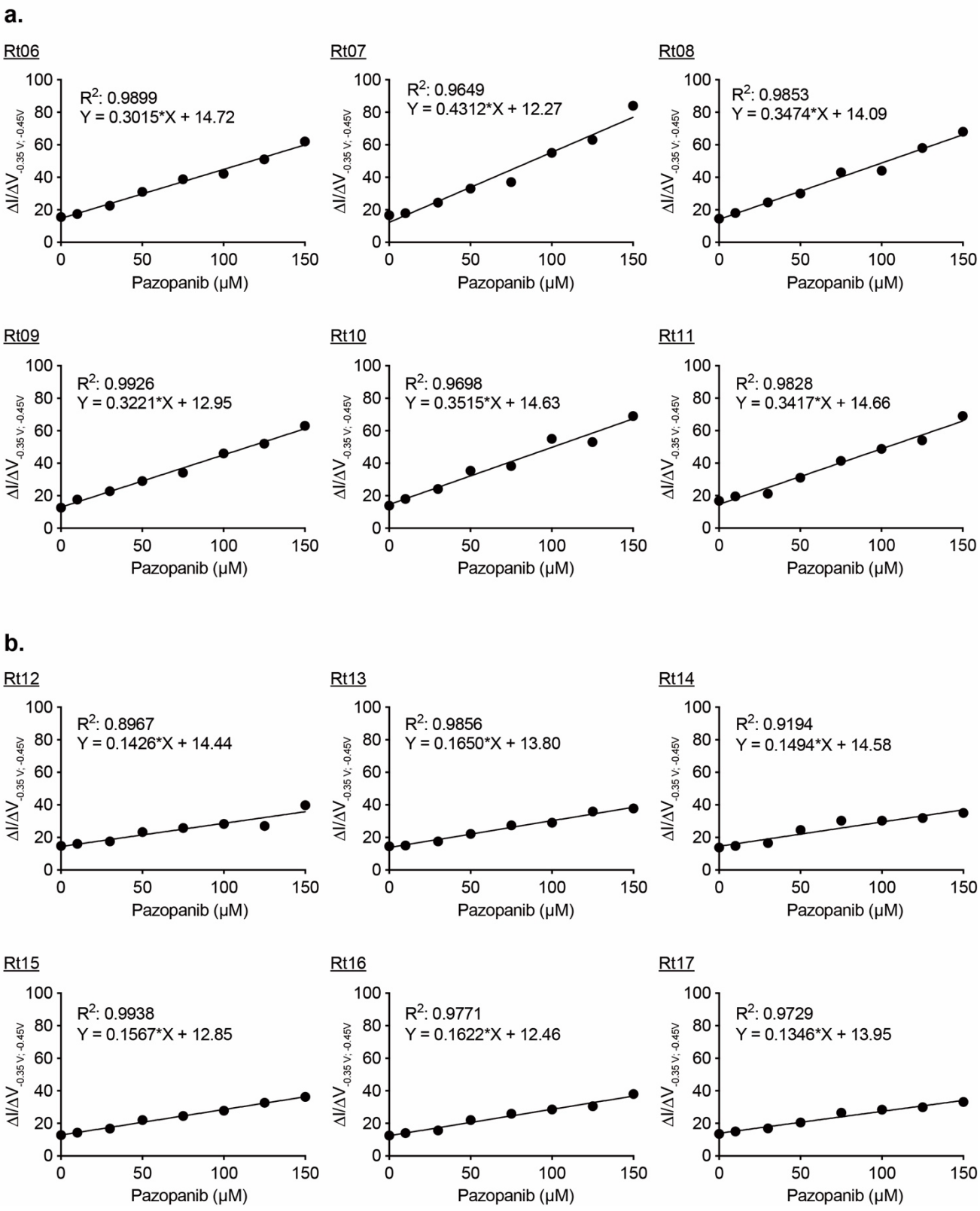

R2: 0.9333
Y = 0.3044*X + 23.74
ΔI/ΔV-0.35 V; -0.45V
Pazopanib (μM)

b.


Pt01
R2: 0.9199
Y = 0.2871*X + 23.86
Pt02
R2: 0.9765
Y = 0.3149*X + 19.98
Pt03
R2: 0.9796
Y = 0.2954*X + 24.11
Pt04
R2: 0.9670
Y = 0.2970*X + 26.70
Pt05
R2: 0.9337
Y = 0.3157*X + 27.83
Pt06
R2: 0.9873
Y = 0.3092*X + 20.37
Pt07
R2: 0.9723
Y = 0.2940*X + 22.74
Pt08
R2: 0.9503
Y = 0.3217*X + 24.32
ΔI/ΔV-0.35 V; -0.45V
Pazopanib (μM)

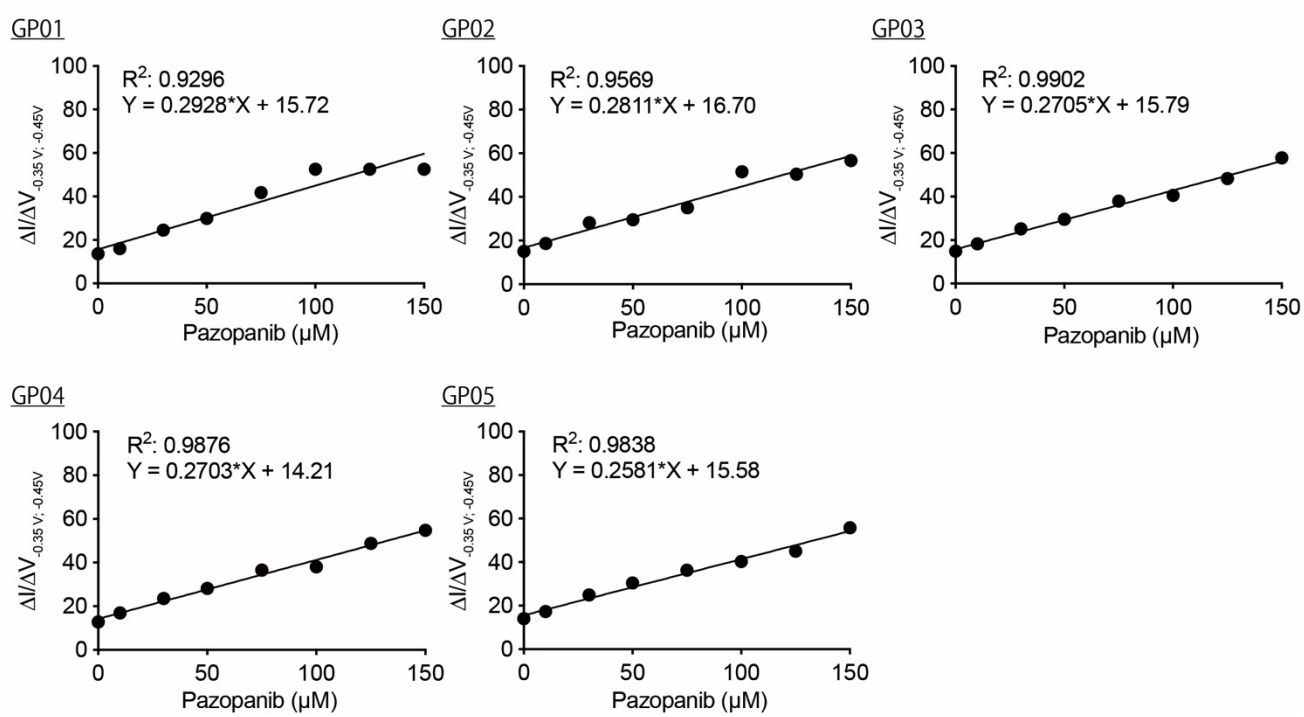
Supplementary Figure 8 Calibration curves for measurements of pazopanib concentrations in the plasma of systemically treated patients.

For each of the eight patients enrolled in the clinical trial, a calibration curve was built by means of the tabletop system containing a BDD chip (chip ID: F) with a mixture of pazopanib (0−150 μM) and plasma isolated from whole blood collected before the oral drug administration. The procedure and protocol were identical to those in **Figure 3a−c**. *Panels **a*** and ***b*** are 'general' and individual calibration curves, which were used in **Figures 5a** and **6,** respectively (see the *main text*). Patient IDs are given above the *panels*.


a.
Rt06
R2: 0.9899
Y = 0.3015*X + 14.72
Rt07
R2: 0.9649
Y = 0.4312*X + 12.27
Rt08
R2: 0.9853
Y = 0.3474*X + 14.09
Rt09
R2: 0.9926
Y = 0.3221*X + 12.95
Rt10
R2: 0.9698
Y = 0.3515*X + 14.63
Rt11
R2: 0.9828
Y = 0.3417*X + 14.66
b.
Rt12
R2: 0.8967
Y = 0.1426*X + 14.44
Rt13
R2: 0.9856
Y = 0.1650*X + 13.80
Rt14
R2: 0.9194
Y = 0.1494*X + 14.58
Rt15
R2: 0.9938
Y = 0.1567*X + 12.85
Rt16
R2: 0.9771
Y = 0.1622*X + 12.46
Rt17
R2: 0.9729
Y = 0.1346*X + 13.95
ΔI/ΔV-0.35 V,-0.45V
Pazopanib (μM)

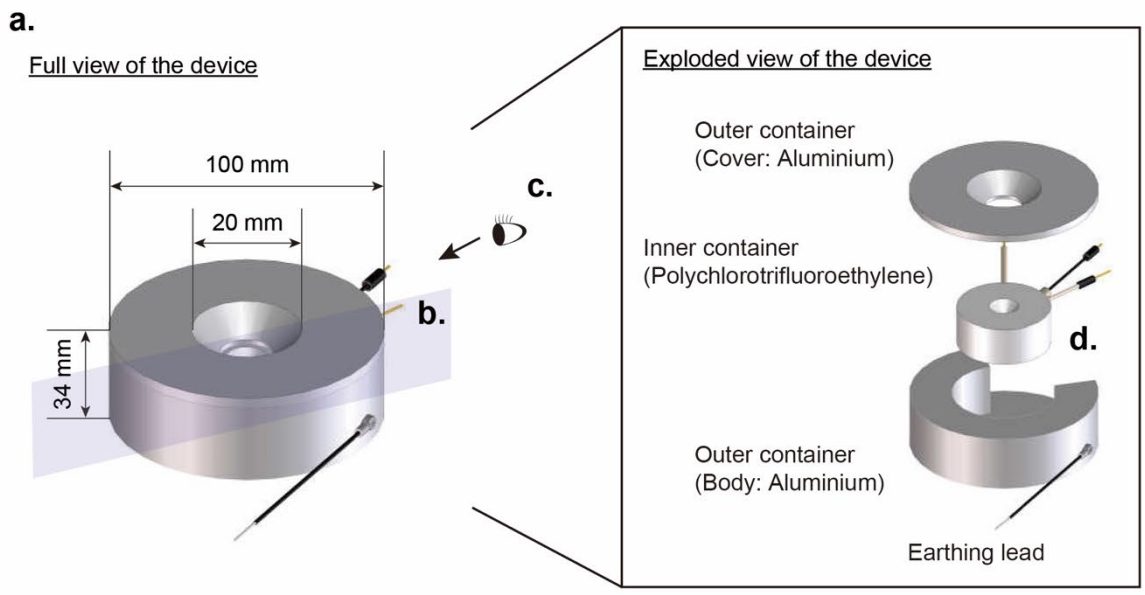
Supplementary Figure 9 Calibration curves built using plasma samples from multiple rats by means of different BDD chips.

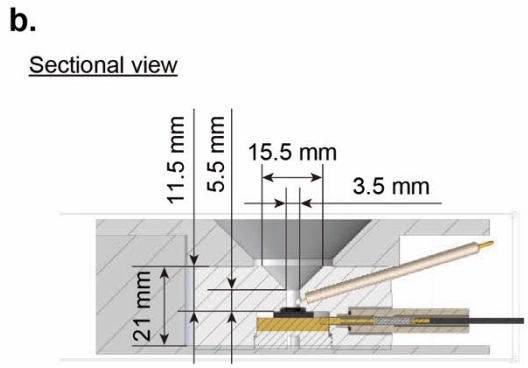
In these series of experiments, whole blood was collected from six different rats, and samples of each isolated plasma were spiked with different amounts of pazopanib (0−150 μM). Then, the plasma samples were utilised to construct calibration curves with the help of the tabletop system containing different BDD chips (chip D for ***a*** and chip E for ***b***). The procedure and protocol were the same as those in **Figure 3a−c**. Animal ID numbers and slope and R^2 values of the regression line are shown in each *panel*. The slope values are plotted in **Figure 6**.

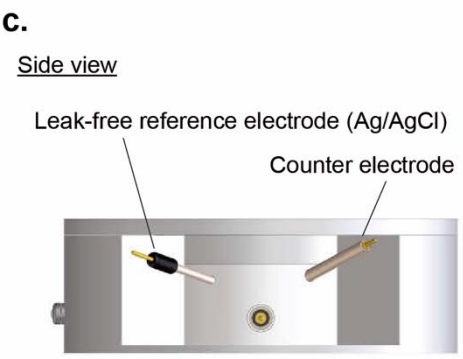


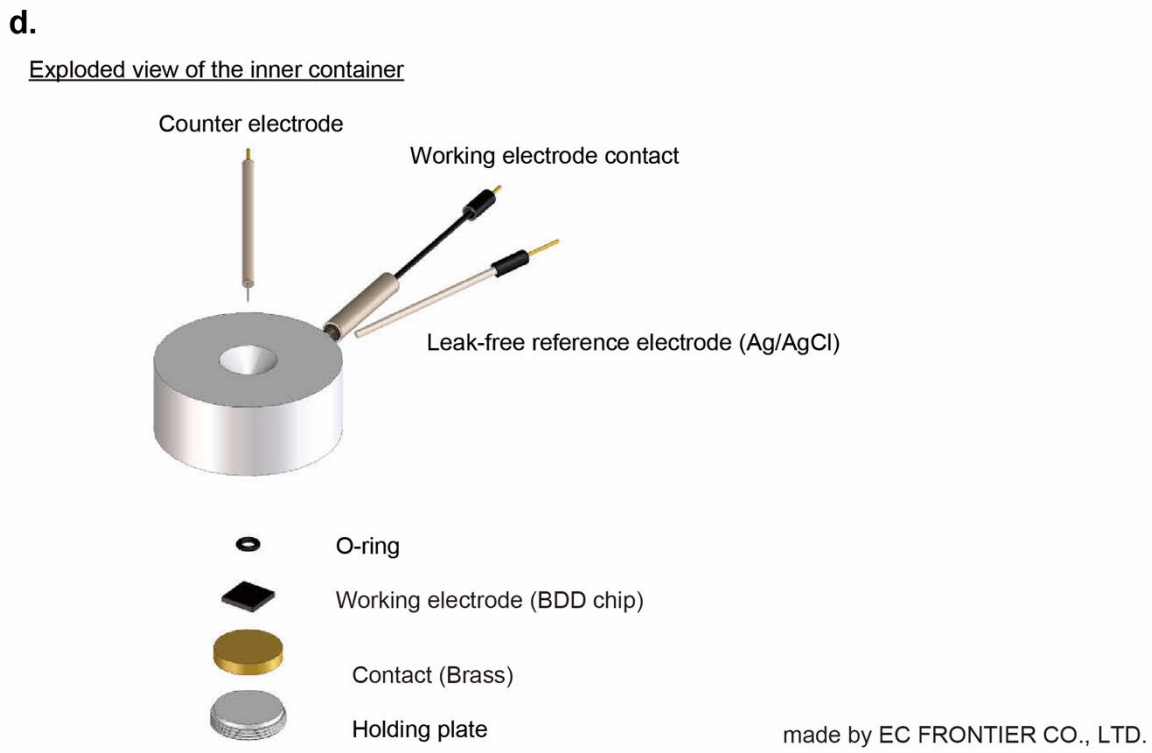
Supplementary Figure 10 Validation of a BDD chip by means of guinea pig plasma containing pazopanib.

In this series of experiments, which involved the tabletop system equipped with BDD chip F, the electrochemical measurement and analysis with the method described in **Figure 3a−c** were carried out for each of five guinea pigs to construct the calibration curves. Animal ID numbers and slope and R^2 values of the regression line are shown in each *panel*. The slope values are plotted in **Figure 6**.


a.
Full view of the device
100 mm
20 mm
34 mm
b.
c.
Exploded view of the device
Outer container
(Cover: Aluminium)
Inner container
(Polychlorotrifluoroethylene)
d.
Outer container
(Body: Aluminium)
Earthing lead


b.
Sectional view
11.5 mm
5.5 mm
15.5 mm
3.5 mm
21 mm


c.
Side view
Leak-free reference electrode (Ag/AgCl)
Counter electrode


d.
Exploded view of the inner container
Counter electrode
Working electrode contact
Leak-free reference electrode (Ag/AgCl)
O-ring
Working electrode (BDD chip)
Contact (Brass)
Holding plate
made by EC FRONTIER CO., LTD.

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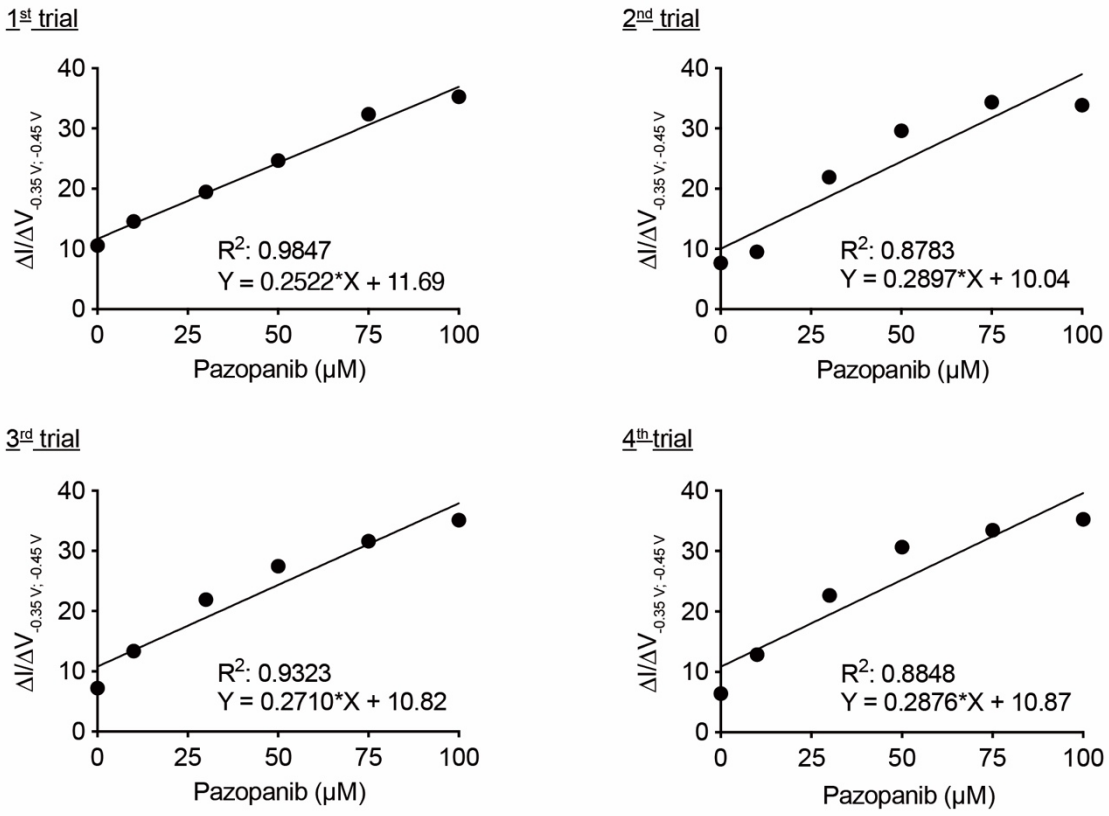
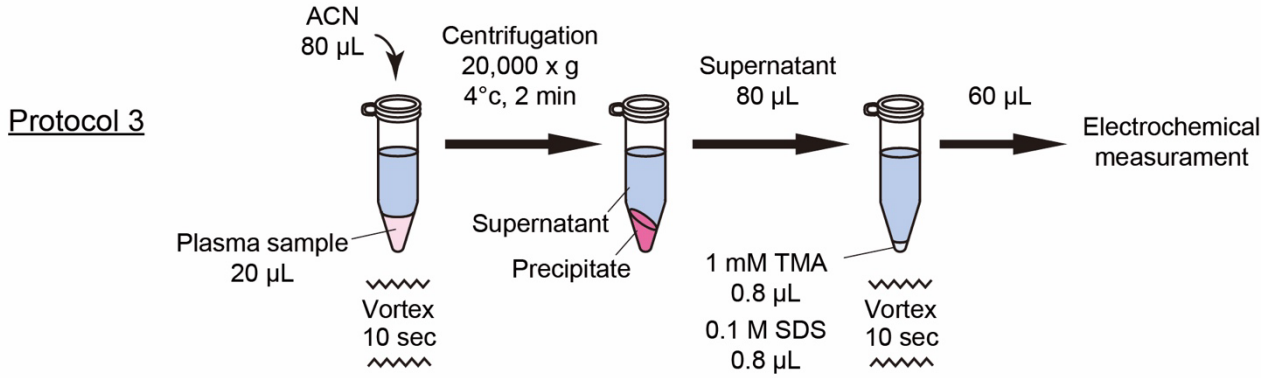
Supplementary Figure 11 The blueprint of the portable system for drug monitoring.

The outward appearance of the handheld disk-shaped measurement device is presented in the *left panel* of ***a*** (see also **Figure 7a**). This device is composed of an aluminium holder containing a BDD chip, an aluminium container, and a copper earthing lead (*right boxed panel*). Sectional and side views are also displayed in *panels* ***b*** and ***c***, respectively, along with the size of the compartments. In ***d***, the components of the holder are shown.

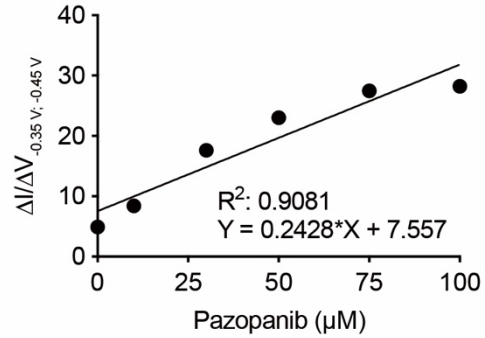
a.


1st trial
2nd trial
3rd trial
4th trial
ΔI/ΔV-0.35 V; -0.45 V
Pazopanib (μM)
R2: 0.9847
Y = 0.2522*X + 11.69
R2: 0.8783
Y = 0.2897*X + 10.04
R2: 0.9323
Y = 0.2710*X + 10.82
R2: 0.8848
Y = 0.2876*X + 10.87

b.


Protocol 3
ACN
80 μL
Centrifugation
20,000 x g
4°c, 2 min
Supernatant
80 μL
60 μL
Electrochemical
measurament
Plasma sample
20 μL
Vortex
10 sec
Supernatant
Precipitate
1 mM TMA
0.8 μL
0.1 M SDS
0.8 μL
Vortex
10 sec

c.


ΔI/ΔV-0.35 V; -0.45 V
Pazopanib (μM)
R2: 0.9081
Y = 0.2428*X + 7.557

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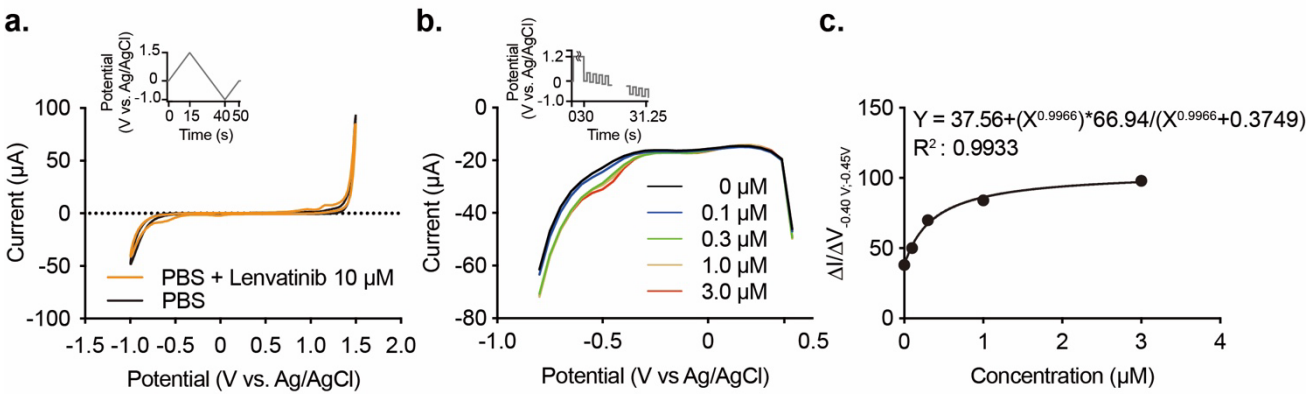
Supplementary Figure 12 Individual data on pazopanib measurements with the portable system.

In *panel* ***a***, pazopanib at different concentrations was mixed with plasma obtained from a healthy rat (animal ID: Rt18): as shown in **Figure 3a–c**, the plasma samples containing the drug at 0 to 100 μM were electrochemically assayed with a boron-doped diamond (BDD) chip (chip ID: H) in the portable system (**Figure 7a** and **Supplementary Figure 11**). This series of experiments was conducted four times (1st through 4th trial). From the raw Osteryoung square wave stripping (OSWS) voltammograms, a $\Delta I/\Delta V_{-0.35\ V;-0.45\ V}$ value at each pazopanib concentration was extracted and plotted against the concentration in *the panels*. Slope and R^2 values of the regression line are presented in each *panel*. The slope values are displayed in **Figure 7b**. *Panel* ***b*** describes the sample preparation procedures for the assays in **Figure 7c**. In this 'Protocol 3', rat plasma (20 μL) was mixed with 80 μL of acetonitrile (ACN) on a vortex mixer for 10 s and centrifuged at 20,000 × *g* for 2 min (4 °C). Then, 80 μL of the supernatant was transferred to a tube that already contained 0.8 μL of 0.1 M SDS and 0.8 μL of 1 mM TMA, followed by vortexing for 10 s. Finally, 60 μL of this sample was subjected to electrochemical measurement. *Panel* ***c*** shows the calibration curve generated by means of commercially available rat plasma (chip ID: I). The protocol was identical to the one in the experiments in *panel* ***a***. The calibration curve was used for the analysis in **Figure 7c**.



Supplementary Figure 13 Electrochemical analysis of lenvatinib.

a, The cyclic voltammogram. Either phosphate-buffered saline (PBS; *black curve*) alone or 10 μM lenvatinib dissolved in PBS (*orange curve*) was examined in the tabletop system (BDD chip ID: J) by the potential protocol described in the *inset* (sweep rate: 0.1 V s^{-1}, potential window: −1.0 to 1.5 V, and initial potential: 0 V versus Ag/AgCl).

b, An Osteryoung square wave stripping (OSWS) voltammogram. Rat plasma spiked with lenvatinib at different concentrations described in the *panel* was tested in the tabletop system (BDD chip ID: J). The potential protocol (*inset*) involved the following parameters: deposition potential, 1.2 V; deposition time, 30 s; potential range, –0.8 to 0.4 V; ΔE, 50 mV; square-wave frequency, 20 Hz; and pulse amplitude, 125 mV.

c, A calibration curve. The $\Delta I/\Delta V_{-0.35\ V;-0.45\ V}$ value at each drug concentration was extracted from the result in *panel* ***b*** and plotted. The data were fitted to the four-parameter logistic regression model, and the Hill slope and R^2 value of the nonlinear regression sigmoidal curve are indicated.

Supplementary Tables

Supplementary Table 1 Optimised parameters for mass-spectrometric analysis

Parameter	Pazopanib	Haloperidol (internal standard)
Turbo ion spray temperature	550 °C	550 °C
Ion spray voltage	−4500 V	5500 V
Curtain gas	40 psi	40 psi
Collision gas	9 psi	9 psi
Ion source gas 1	70 psi	70 psi
Ion source gas 2	60 psi	60 psi
Declustering potential	−140 V	120 V
Entrance potential	−10 V	10 V
Collision energy	−42 V	31 V
Collision cell exit potential	−10 V	25 V

Supplementary Table 2 Lists of chip, animal, and patient IDs

Display item	BDD chip ID	Rat ID	Patient ID	Guinea pig ID
Figure 1	A	–	–	–
Figure 2	C	Rt27	–	–
Figures 3b, 3c, and 6 Supplementary Figures 5 and 9a	D	Rt06−11	–	–
Figures 3d and 6 Supplementary Figure 9b	E	Rt12−17	–	–
Figures 4 and 6 Supplementary Figures 6 and 7	F	Rt01−05 Rt23−26	–	–
Figures 5 and 6 Supplementary Figure 8	F	–	Pt01−08	–
Figure 6 Supplementary Figure 10	F	–	–	GP01−05
Figure 7b Supplementary Figure 12a	H	Rt18	–	–
Figure 7c Supplementary Figure 12b	I	Rt19−22	–	–
Supplementary Figure 2	B	–	–	–
Supplementary Figure 4	G	–	–	–
Supplementary Figure 13	J	Rt28	–	–

References for Supplementary Figure Legends

1. Muzyka, K., *et al.* Boron-doped diamond: current progress and challenges in view of electroanalytical applications. *Analytical Methods* **11**, 397-414 (2019).