Supporting Information

Tetrapyrrolic Pigments from Heme- and Chlorophyll Breakdown are Actin-Targeting Compounds

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# Supporting Information

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1. Material and Methods

1.1 General

Chemicals and Materials

Acetic acid (AcOH), HPLC grade acetonitrile (ACN), dichloromethane (DCM), dimethylsulfoxide (DMSO), hydrochloric acid (HCl), HPLC grade methanol (MeOH), sodium hydroxide (NaOH), and silica gel 60 were obtained from VWR (Ismaning, Germany) and ultra-pure water (18MΩ.cm⁻¹) from a Millipore S.A.S. Milli-Q Academic system (18,2 Ω cm⁻¹, Molsheim, France). Adenosine 5’-triphosphate (ATP), ammonium acetate (NH₄AcO), bovine serum albumin (BSA), calcium chloride (CaCl₂), catalase, d₆-DMSO, ethylene glycol-bis(2-aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA), D-glucose, glucose oxidase from Aspergillus Niger, Hoechst 33342, imidazole, methylcellulose, β-mercaptoethanol, potassium phosphate dibasic (K₂HPO₄), potassium phosphate monobasic (KH₂PO₄), rhodamine-phalloidin, Tris-HCl, pyronin Y, pyridine, zink acetate (Zn(AcO)₂), and 96 well black polystyrene half-area microplates were purchased from Sigma-Aldrich (Taufkirchen, Germany). SepPak Plus C18 cartridges were obtained from Waters Associates (Milford, USA). DMEM medium was purchased from PAN-Biotech (Aidenbach, Germany); fetal calf serum (FCS) from PAALaboratories (Pasching, Austria), and Triton X-100, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (MTT reagent) and FluorSave® reagent mounting medium were obtained from Merck (Darmstadt, Germany). Magnesium chloride (MgCl₂) and glycerol were purchased from AppliChem (Darmstadt, Germany). Dithiothreithol (DTT) was purchased from SERVA Electrophoresis (Heidelberg, Germany). Crystal violet, potassium chloride (KCl), and sodium dodecyl sulfate (SDS) were purchased from Carl Roth (Karlsruhe, Germany). Paraformaldehyde (PFA) was obtained from Thermo Fisher Scientific (Waltham, USA). Coomassie Brilliant Blue R-250 staining solution was purchased from Bio-Rad (Munich, Germany). Bilirubin and biliverdin from Cayman chemicals were purchased from biomol (Hamburg, Germany).

Cell culture

The human bladder cancer cell line T24 and the cervical cancer cell line Hela were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ; Braunschweig, Germany) and maintained in DMEM medium supplemented with 10% fetal calf serum (FCS). Cells were cultured at 37°C under 5% CO₂ atmosphere with constant humidity.

Plant Material

Senescent leaves of the Katsura tree (Cercidiphyllum japonicum) were collected in the Maria-Ward-Straße, Munich (48°09'41.0"N 11°29'58.8"E). Prof. Susanne S. Renner (Department of Systematic Botany and Mycology, Faculty of Biology, University of Munich) determined the identity of the plant material; the voucher specimen of Cercidiphyllum japonicum (Moser & Karg 3) has been deposited in the Munich herbarium (acronym M).
Chromatography

i) Analytical HPLC: An Agilent 1260 Infinity II LC system was used with a 1260 Infinity Degasser, a 1260 Series quaternary pump and 1260 Series diode array detector; Merck LiChrospher® 100 RP-18 (5 µm) LiChroCART® 125-4, protected by a Merck LiChrospher® 100 RP-18 (5 µm) LiChroCART® 4-4 i.d. pre-column; injection volume: 100 µL (unless stated otherwise). Solvent system: mobile phase A = NH₄AcO buffer 10 mM pH 7, B = ACN, flow 0.5 mL/min; Solvent composition: 0-2 min 5% B, 2-17 min 5% to 100% B, 17-20 min 100% B, 20-22 min 100% to 5% B. Solvent system for bilirubin detection: mobile phase A = 0.1% formic acid, B = ACN, flow 0.5 mL/min; Solvent composition: 0-2 min 40% B, 2-10 min 40% to 75% B, 10-18 min 75% to 95% B, 18-28 min 95% B, 28-35 min 95% to 40% B. Data were processed with OpenLab CDS Data Analysis 2.3.

ii) Semi-preparative HPLC: Büchi Pure C-830 with prep HPLC pump 300 bar, fraction collector, and prep sample injection valve. Gynkotek LC-System with manual sampler, M480 pump, Phenomenex DG-301 online degasser, Gynkotek UVD 640 diode array detector and a Rheodyne injection valve with 5 mL loop. Column: Supelco Ascentis® C18, 5 µm, 15 cm x 10 mm, with a Phenomenex pre-column ODS 9 x 16 mm; mobile phase A = NH₄AcO buffer 10 mM pH 7, B = MeCN, flow 2.5 mL/min; solvent composition: 0-2 min 12% B, 2-12 min 12% to 20% B, 12-30 min 20% to 80% B, 30-40 min 80% to 100% B. Data were processed with Gynkosoft 5.50 or Büchi Pure software 1.5.

Spectroscopy

UV-Vis: Thermo Spectronic Genesys 5 (336001) UV-Visible spectrophotometer. Concentrations of PxB were calculated using log ε (426 nm) = 4.51[1], concentrations of PrB were calculated using log ε (523 nm) = 4.56[2].

HR-MS were measured at the MS facility of the Department of Chemistry, University of Munich. Data were processed using Xcalibur. NMR spectra were recorded on an Avance III HD 500 MHz NMR spectrometer from Bruker BioSpin equipped with a CryoProbe™ Prodigy broadband probe holder. NMR data were analyzed with Mestre Nova.

Compounds

During all handling steps, the material was protected from light and temperatures above 37 °C were avoided, if not stated otherwise.

Bilirubin and biliverdin were dissolved immediately before use in 0.2 N NaOH or 0.1 N NaOH for cell assays, respectively, or in DMSO for all in vitro assays to a final stock concentration of 10 mM or 20 mM and further diluted as indicated.

PxB was synthesized from its precursor PleB, which was isolated from senescent leaves of Katsura tree according to the published protocol with minor modifications [2-3]. Briefly, 500 g of senescent leaves of Cercidiphyllum japonicum were extracted by hot water extraction with 1.5 L of boiling water. The aqueous mixture was extracted twice with 1:1 volumes of DCM. The organic phases were combined
and the solvent was evaporated. The dry residue was dissolved in 20 mL of DCM, 10 g silica were added and the solvent was carefully evaporated under vacuum. The silica was transferred into a beaker and illuminated under a tungsten light bulb overnight while stirring. The oxidation of PleB to PxB was controlled by analytical HPLC. After eluting the mixture with MeOH and filtering it through a paper filter, PxB and the remaining PleB were isolated by semi-preparative HPLC. The purity of PxB was confirmed by analytical HPLC. Pure PxB was dried, diluted with 5 mL of ACN/potassium phosphate buffer (pH 2.5) 20/80 and stirred overnight in the dark. The solution was first applied to a Sep-Pak-C18 column (5g), PxB was eluted with ACN and solvent was evaporated before dissolving the product in DMSO and storing it at -20°C until further use. Yields were typically in the range of 5 to 10 mg of pure PxB.

PrB was synthesized from PxB according to the protocol of Li et al with modifications. 27 mg of PxB (42.06 µmol) was dissolved in 15 mL DMF, and 39.67 mg of Zn(AcO)₂ (216.21 µmole, 5.1 eq) and 3 mL of pyridine were added. The mixture was stirred overnight under air and in the dark until the dark yellow solution turned dark blue. After 20 h, the mixture was diluted with 50 mL of water and extracted three times with 50 mL of DCM each. DCM was evaporated before 30 mL of ACN, 30 mL of PBS (pH 4.7) and 50% (v/v) AcOH were added until a pH value of 2.5 was reached. While the solution was stirred for 1 h, the colour changed from dark blue to dark red. Next, the mixture was extracted with equal volumes of DCM. The solvent was again evaporated, the residue was dissolved in MeOH/potassium phosphate buffer (pH 7) 20/80 and applied to a Sep-Pak-C18 cartridge (5g). PrB was eluted with MeOH and the purity was confirmed by analytical HPLC. The methanolic solution was dried and pure PrB was diluted with 5 mL of ACN/potassium phosphate buffer (pH 2.5) 20/80 and stirred overnight in the dark. The solution was applied to a Sep-Pak-C18 cartridge (5g), PrB was eluted with ACN and solvent was evaporated before dissolving the obtained PrB with DMSO. Yields were typically in the range of 90 to 93%.

Analytical and spectroscopic data of PxB and PrB are consistent with the cited literature.

**Spectroscopic data**

**PxB.** UV/Vis online spectrum (nm, rel λ): 212 (1.00), 244 (0.58), 312 (0.70), 424 (0.94) nm. HR-ESI-MS: m/z calculated (C₃₅H₃₉O₈N₄) = 643.27624 [M+H⁺]; m/z found = 643.27652 (Δ = 0.28 ppm).

²¹H-NMR (500 MHz, d₆-DMSO): δ [ppm] = 2.03 (s, H₃C7¹); 2.06 (s, H₃C13¹); 2.15 (s, H₃C17¹); 2.17 (s, H₃C2¹); 2.20 (m, H₃C12²); 2.36 (m, H₃C12³); 2.46 (m, H₃C3¹); 2.54 (m, H₃C12¹); 3.34 (m, H₃C3³); 3.66 (m, H₃C8⁵); 3.77 (m, H₃C5); 3.86 (m, H₂C8²); 4.80 (m, H₁C₁₀); 5.31 (m, H₂C₆¹); 6.06 (m, H₁C₁₅); 6.20 (m, H₂C₁₈²); 6.57 (ddd, J = 11.4/17.6, H₁C₁₈¹), 9.46 (m, H₂C₂₀), 10.07 (s, H₂N₂₄).

**PrB.** UV/Vis online spectrum (nm, rel λ): 208 (0.59), 314 (0.72), 524 (1.00) nm. HR-ESI-MS: m/z calculated (C₃₅H₃₇O₈N₄) = 641.26059 [M+H⁺]; m/z found = 641.26129 (Δ = 0.7 ppm).

²¹H-NMR (500 MHz, d₆-DMSO): δ [ppm] = 2.09 (s, H₃C13¹), 2.12 (s, H₃C7¹), 2.19 (s, H₂C2¹), 2.20 (s, H₃C17¹), 2.15 (m, H₂C12²), 2.53 (m, H₂C3¹), 3.07 (s, H₂C₁₂¹), 3.61 (s, H₂C₈²), 4.11 (d, J = 4.0, H₂C₅),
5.04 (s, HC8²), 5.49 (dd, J = 2.38/11.58, HaC18²), 6.30 (dd, J =2.4/17.59, HaC18²), 6.15 (s, HC15), 6.65 (dd, J = 11.59/17.64, HC18¹), 9.51 (s, HC20), 10.00 (s, HN24).

1.2 Immunofluorescence staining

T24 or Hela cells (2 x 10⁴ cells/well) were seeded in 8-well ibiTreat μ-slides (ibidi GmbH, Gräfelfing, Germany) and allowed to adhere overnight. Cells were treated with the indicated concentrations of compounds or vehicle controls and incubated for 4 h at 37°C. Afterwards, cells were washed twice with PBS+ (PBS including Ca²⁺, Mg²⁺), fixed with 4% (v/v) paraformaldehyde in PBS for 10 min and permeabilized with 0.2% (v/v) Triton X-100 in PBS for 10 min. Antibody solution containing rhodamine-phalloidin (1:400) to stain F-actin and Hoechst 33342 (1:50) to label nuclei in 1% bovine serum albumin (BSA) in PBS was added and incubated for 60 min. Cells were finally washed three times with PBS for 10 min and covered with FluorSave™ reagent mounting medium and glass coverslips. Images were collected with a Leica SP8 confocal laser scanning using Leica LAS X software and analyzed with ImageJ 1.45s.

1.3 Wound-healing assay

Cell migration was analyzed in a wound healing assay. T24 and Hela cells were seeded into 96 well plates (3 x 10⁴ cells/well) and allowed to adhere until a cell density of 100% confluence was reached (for experiments with PxB and BR, plates were coated with 0.001% collagen G in PBS). The monolayers were wounded in a 96-well format with a custom made tool, washed with PBS+ and treated with the indicated concentrations of compounds or vehicle controls prepared in DMEM medium with FCS. DMEM medium without FCS served as a negative control and DMEM medium with serum as positive control. Cells were allowed to migrate until first contacts of cells in the positive control were observed (16-24 h). Next, medium was discarded; cells were washed with PBS+, stained with 0.5% crystal violet solution for 10 min and washed with water. After drying overnight, images were taken on a Leica DM1 microscope and a Leica MC120 HD camera with a 4x phase contrast objective (Leica Microsystems, Wetzlar, Germany) and the uncovered area was analyzed using a custom-written program in Matlab R2017a. Migration was quantified as the percentage of the uncovered area normalized to the negative control, which was set to 100%.

1.4 MTT assay

Cell viability was determined using an MTT assay in parallel to the migration assay to exclude cytotoxic effects of the different compounds at the applied concentrations. As described above, 3x10⁴ T24 or Hela cells were seeded in 96 well plates. After incubating overnight, cells were treated with the indicated concentrations of compounds or vehicle controls and incubated in parallel to the scratch assay. 2 h before the end of stimulation time of the scratch assay, 10 µL of MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) were added to each well and cells were incubated for 1 h at 37°C. Next, 190 µL of DMSO was added and the cells were again incubated for 1 h at room temperature while shaking and protected from light before absorbance was measured at
550 nm using a Tecan SpectraFluor plus microplate reader. Results were normalized to the vehicle control, which was set to 100%.

1.5 Actin polymerization and depolymerization assay

The actin polymerization or depolymerisation assays were performed by using pyrene labeled actin (10%), F-Actin BufferKit and PolyMix (10×stock), purchased from Hypermol (Bielefeld, Germany), according to the manufacturer's instructions. Briefly, pyrene labeled actin (10%) was diluted with H2O to a concentration of 1mg/mL (24 μM) or 1.1mg/mL (26 μM) stock solution and subsequently centrifuged at 55,000xg and 4 °C for 60 min to remove any aggregates from freeze-drying.

**Polymerization:**

For the polymerization assay, a 1mg/mL (24 μM) pyrene actin stock solution was prepared. Actin polymerization solution consisting of 20 μL of H2O, 10 μL of 10 mM MgCl2, 5 μL of F-actin buffer (100 mM Imidazole-Cl pH 7.4, 10 mM ATP), as well as 5 μL of either DMSO or the respective compound, was prepared in a 96-well black polystyrene half-area microplate. 10 μL/well of pyrene labeled actin was added and pyrene fluorescence was immediately monitored every 20 s over 1 h with a Tecan Infinite® 200 PRO fluorescence plate reader at 360 nm excitation and 400 nm emission wavelength. Baseline fluorescence intensities were subtracted from total fluorescence values.

**Depolymerization:**

For depolymerization, pyrene labeled F-actin was obtained by incubating pyrene labeled actin (1.1 mg/ml, 26 μM) in 10× PolyMix buffer (1 M KCl, 0.1 M imidazole pH 7.4, 10 mM ATP, 20 mM MgCl2) at room temperature for 1 h. This yields a 24 μM pyrene labeled F-actin stock solution. Before use, F-actin was diluted 1:1 with 1× PolyMix buffer, resulting in a 12 μM sample solution. Depolymerization solution (consisting of 25 μL of 1× PolyMix buffer and 5 μL of either DMSO or compound) was added into a 96-well black polystyrene half-area microplate immediately before rapid addition of 20 μL of pyrene labeled F-actin (12 μM). Depolymerization was monitored by measuring pyrene fluorescence every 20 s over 2 h with a Tecan Infinite® 200 PRO fluorescence plate reader at 360 nm excitation and 400 nm emission wavelength. Initial fluorescence values of each condition were subtracted from total fluorescence values.

1.6 TIRF assay

The TIRF (Total Internal Reflection Fluorescence) microscopy assay was performed according to published protocols with minor modifications.[4] Flow cells (containing 15-20 μL of fluid) were made as a sandwich of a cover slip (22 × 22 mm), two parafilm strips forming an approximately 5 mm wide channel and a glass slide (76 × 26 mm).

For TIRF microscopy, flow-cell chambers were used with the cover slip down, facing the objective lens, and slide up. Solutions were loaded directly into the chamber via capillary action. Labeled actin was prepared by mixing Atto488-actin and actin 1:1 (v/v) from rabbit skeletal muscle (Hypermol, Bielefeld, Germany). α-actinin from turkey gizzard smooth muscle (Hypermol, Bielefeld, Germany).
Germany) was prepared by adding ultrapure water to obtain a 1 mg/mL stock solution and used as tethering protein.

**Nucleation assay**

Flow cell chambers were freshly passivated by incubation with 25 μL of 1% (w/v) BSA in PBS for 10 min. 25 μL of α-actinin (0.1 mg/mL) were then applied for 5 min. In the meantime, Mg-ATP-actin (5 μM) was prepared. Labeled actin (10 μM) was incubated 1:1 with 1/10 volume of 10 × Mg-exchange buffer (2 mM EGTA, 400 μM MgCl₂) and 1:8 with G-buffer (2 mM Tris-HCl, pH 8.0, 0.2 mM CaCl₂, 0.5 mM DTT, 0.2 mM ATP, final pH 7.8) on ice for 5 min to exchange Ca-ATP-actin for Mg-ATP-actin. Actin polymerization was triggered by a 1:1 mixing of Mg-ATP-actin (5 μM) with 2 × TIRF buffer (100 mM KCl, 2 mM MgCl₂, 2 mM EGTA, 30 mM imidazole, 30 mM D-glucose, 40 μg/mL catalase, 400 μg/mL glucose oxidase, 1% methylcellulose, 2% β-mercaptoethanol, final pH 7.4) containing the indicated concentrations of compounds. 30 μL of polymerizing actin was immediately loaded into the flow-cell chamber and placed on the TIRF microscope (Leica Microsystems, Wetzlar, Germany). The number of actin nuclei present in each frame (calculated from the number of filaments) was analyzed by using custom-written programs in Matlab R2017a.

**1.7 Computational methods**

Docking experiments were performed to evaluate the binding of the phyllobilins (PxB and PrB) and the bilins (BR and BV) to actin. The actin structure (PDB ID 4k41) in complex with Kabiramide C (KabC) was prepped in Molsoft’s ICM version 3.9.1 KabC.1. KabC bound to actin was removed from the structure and receptor maps were generated for docking studies encompassing the KabC binding site. Docking of each ligand was performed with the evaluation of multiple conformations for each ligand.

**1.8 G-actin binding assay**

The G-actin binding assay was performed using the ‘Actin-Toolkit G-Actin Binding’ (Hypermol, Bielefeld, Germany), in which G-actin is coupled to SepharoseTM as G-actin beads. To investigate the binding of the compounds to G-actin, supernatants of the G-actin binding assay were analyzed for the respective compounds by HPLC. Agarose beads served as negative control. According to the manufacturer’s instructions, G-actin beads and agarose beads were prepared in MonoMix buffer (Hypermol, Bielefeld, Germany) and 50 μL each were incubated with the different compounds (50 μM) for 1 h at room temperature under agitation in G-actin buffer (20 mM Tris-HCl, 0.2 mM ATP, 0.2 mM CaCl₂, pH 7.9). After incubation, samples were spun at 6,000×g for 4 min and supernatants were collected. The pellets were washed (3~4 times) and washing buffer was collected. After washing, the pellets were incubated with elution buffer (50% ACN, for BR 0.1 N NaOH) for 30 min under agitation and the samples were again centrifuged. The collected elution buffer was analyzed by HPLC. Peak areas of different compounds were analyzed using OpenLab CDS 2.3 software (for PxB detection at 420 nm, for PrB at 520 nm, for BR at 450 nm, and for BV at 320 nm) and peak areas of agarose beads were normalized to values with G-actin beads, which were defined as 1.0.
For further investigating the binding of PxB, PrB, BR, and BV to G-actin in competition with the actin binding protein profilin, G-actin beads and profilin (Hypermol, Bielefeld, Germany) were prepared according to the manufacturer's instructions. G-actin beads were pre-treated with compounds (50 μM) or vehicle control for 1 h at room temperature under agitation. Then profilin (0.667 μM) was added and incubated for 1 h at room temperature under agitation. Samples were spun at 6,000×g for 4 min and 40 μL of supernatant was prepared for an SDS-sample by adding 10 μL of 5x SDS buffer (3.125 M Tris-HCl pH 6.8, 50% glycerol, 5% SDS, 2% DTT, 0.025% pyronin Y, H2O). The pellet was washed, boiled for 2 min at 95°C to release profilin from the beads, and mixed with 25 μL of 1xSDS-sample buffer. Both the supernatant and pellet were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) in electrophoresis buffer (100 V, 21 min then 200 V, 55 min) using a 18% polyacrylamide gel. Gels were stained for 30 min in Coomassie blue solution, washed with water and destained in destaining solution overnight (10% glacial acetic acid, 30% methanol, and 60% distilled water). Images of the gels were captured using a ChemiDoc Imaging System (Bio-Rad Laboratories GmbH). The amount of each protein was quantified by using Image Lab 6.0 Software and normalized to the vehicle control, which was defined as 1.0.

1.9 Determination of $K_d$ values by fluorescence quenching

To characterize the interaction between G-actin and PrB or G-actin and BR, fluorescence measurements were conducted on an FLS 1000 Fluorimeter (Edinburgh Instruments). The emission spectra were recorded using a 450 W continuous wave Xenon lamp. Fluorescence spectra of Atto647 labeled G-actin (Hypermol, Bielefeld, Germany; 200 nM) alone and upon titration with PrB or BR were measured at an excitation wavelength of 635 nm using an excitation and emission band width of 2 nm in a quartz cuvette (Hellma Analytics, Müllheim, Germany). The spectra were measured in 1 nm steps between 640 and 700 nm with 0.5 and 2s dwell time for PrB and BR, respectively, on a photon multiplier tube (PMT 900 detector, Hamamatsu). Fluorescence intensities at the emission maximum of 669 nm were normalized to the initial fluorescence of labeled actin and the quenching activity in % was plotted against the concentration of PrB/BR. To determine the dissociation constant ($K_d$) of PrB, three independent experiments were performed and a one-site-specific binding model was applied for curve fitting using GraphPad Prism 9.1.1.

1.10 Microscale thermophoresis (MST)

For determination of the dissociation constant of PxB binding to G-actin, microscale thermophoresis measurements were conducted using Atto647 labeled G-actin from Hypermol (Bielefeld, Germany). Briefly, 16 1:1 dilutions of the ligand PxB were prepared in G-actin buffer yielding final concentrations from 10 nM to 345 μM. Each ligand dilution was mixed with one volume of labeled protein (final concentration of 71.4 μM). The samples were loaded into Monolith NT.115 Capillaries and MST was measured using a Monolith NT.115 instrument (NanoTemper Technologies) at an ambient temperature of 25°C. Data of three independently pipetted measurements were analyzed using the signal from an MST-on time of 5 s and the $K_d$ was calculated by MO.Affinity Analysis software version 2.3 (NanoTemper Technologies).
1.11 Isothermal calorimetry (ITC)

ITC measurements were performed in order to determine the dissociation constant ($K_d$) of the interaction of G-actin and BV using a MicroCal PEAQ-ITC (Malvern Instruments, Malvern, UK). Rabbit skeletal muscle alpha actin (Hypermol, Bielefeld, Germany) was diluted, centrifuged for 15 min at 4°C and purified by size exclusion chromatography on a Cytiva ÄktaGo system, equipped with a Superdex 200 increase 10/300 column, equilibrated with 20 mM Tris-HCl, 150 mM NaCl, 0.4m M ATP, pH 8.2. Two sequential ITC measurements with 12 injections of a 1 mM solution of BV in the same sample cell with purified G-actin protein solution (50 µM) were performed. After finishing the experiment, a control experiment with buffer in buffer injections was performed keeping all the parameters same. Data sets of both titrations were merged using MicroCal Concat ITC software and merged data were analysed and the $K_d$ was calculated assuming a 1/1 binding model using MicroCal PEAQ-ITC Analysis Software (v121).

1.12 Statistical analysis

Results represent the mean of at least three independent experiments (means ± SEM) performed in at least three replicates, unless stated otherwise. Statistical significance was carried out by two-way analysis of variance with post hoc analysis using Dunnett’s multiple comparison test or an unpaired t-test with Welch’s correction; all statistical analyses were processed with GraphPad Prism 9.1.1.
2. Supplementary Figures

![Supplementary Figures](image-url)
Figure S1. Characterization of tetrapyrroles. HPLC analysis (upper panels) and UV-Vis online spectra (lower panels) of isolated/synthesized PxB (A) and PrB (B), and commercial BR (C) and BV (D). For the HPLC analysis, detection was performed at 320 nm for PxB, PrB and BV and at 450 nm for BR.
Figure S2. Impact of tetrapyrroles in wound healing assays. Cell migration of Hela cells treated with increasing concentrations of PxB, PrB, BR, BV, and vehicle control (vh ctrl) analyzed by a scratch wound-healing assay. Treatment with cell medium containing FCS served as a negative control and medium without FCS as a positive control. (Upper panels) Bar graphs showing the relative scratch gap normalized to the control without FCS. The bars present the mean±SEM of three independent experiments performed in triplicates at 37°C. (The significance was analyzed using a one-way ANOVA followed by Dunnett’s multiple comparison test, *P < 0.05, **P < 0.01, ***P < 0.001). (Lower panels) Representative images for the scratch analysis collected 16-24 hrs after generating the scratch are shown. Scale bar: 1 mm.
Figure S3. Influence of tetrapyroles on cell viability. Cell viability assay of Hela (A) and T24 (B) cells treated with indicated concentrations of phyllobilins and bilins analyzed by a MTT assay. The percentage of viable cells were normalized to the vehicle control (vh ctrl). Bars show the mean±SEM of three independent experiments performed in triplicates. Significance was calculated using a one-way ANOVA followed by Dunnett’s multiple comparison test, *P >0.12 = not significant (ns).
Figure S4. Influence of tetrapyrroles on the cellular cytoskeleton. Immunofluorescence staining of Hela and T24 cells treated with the indicated concentrations of BR and BV for 4 h. Nuclei were stained with Hoechst (shown in blue) and F-actin with rhodamine-phalloidin (shown in red). Scale bar: 50 µm. Representative images of three independent experiments are shown.
Figure S5: 2D ligand interaction map from the docking analysis of PrB binding to G-actin. The structure of PrB with hydrophobic regions shaded in green, hydrogen bond acceptors shaded in blue, and hydrogen bonds are shown as dashed arrows. The accessible surface areas are illustrated by grey parabolas and as broken thick lines around the ligand. Residues in van der Waals contact are coloured grey and the size of residue ellipse indicates the strength of the contact.
**Figure S6. Competitive G-actin binding assay with compounds and profilin.** G-actin beads were pre-treated with compounds or a vehicle control for 1h before profilin was added. Beads were centrifuged, the supernatant (sn) was collected and the beads thoroughly washed. Only profilin that was bound to G-actin was co-precipitated in the pellet. Pellets were solubilised by boiling to release the bound profilin. Samples were analysed by SDS- PAGE followed by a Coomassie staining. The shown data are representative gels taken from three independent experiments.

**Figure S7. Fluorescence titration experiments of PrB with G-actin.** Fluorescence spectra (Ex. 635 nm; Em. 640-700 nm) were recorded while titrating with increasing concentrations of PrB (0.25 µM – 110 µM) to a solution of Atto647 labeled G-actin (0.2 µM) in G-actin buffer. Three independent experiments were performed and spectra taken from one representative experiment are shown.
Figure S8. Fluorescence titration experiments of BR with G-actin. Fluorescence spectra (Ex. 635 nm; Em. 640-700 nm) were recorded while titrating with increasing concentrations of BR (0.5 µM – 42.75 µM) to a solution of Atto647 labeled G-actin (0.2 µM) in G-actin buffer. Three independent experiments were performed and spectra taken from one representative experiment are shown.

Figure S9. MST interaction analysis of PxB and Atto647 labeled G-actin. MST measurements were carried out with labeled G-actin protein solution (71.4 nM) and increasing concentrations of ligand PxB (0.01 µM to 345.12 µM) in G-actin buffer. MST traces presented are representative traces taken from one of three independent experiments.
Figure S10. Direct binding of BV to G-actin analyzed by ITC. ITC trace of two subsequent titrations of a 1 mM solution of ligand BV into the same sample cell with purified G-actin protein solution (50 µM).

3. References

[1] S. Moser, M. Ulrich, T. Muller, B. Krautler, Photochemical & photobiological sciences 2008, 7, 1577-1581.
[2] C. Li, M. Ulrich, X. Liu, K. Wurst, T. Müller, B. Kräutler, Chemical Science 2014, 5, 3388-3395.
[3] C. A. Karg, P. Wang, F. Kluibenschedl, T. Müller, L. Allmendinger, A. M. Vollmar, S. Moser, European Journal of Organic Chemistry 2020, 2020, 4499-4509.
[4] aD. Breitsprecher, A. K. Kiesewetter, J. Linkner, J. Faix, in Chemotaxis: Methods and Protocols (Eds.: T. Jin, D. Hereld), Humana Press, Totowa, NJ, 2009, pp. 401-415; bL. K. Doolittle, M. K. Rosen, S. B. Padrick, in Adhesion Protein Protocols (Ed.: A. S. Coutts), Humana Press, Totowa, NJ, 2013, pp. 273-293.
[5] R. Abagyan, M. Totrov, D. Kuznetsov, Journal of Computational Chemistry 1994, 15, 488-506.