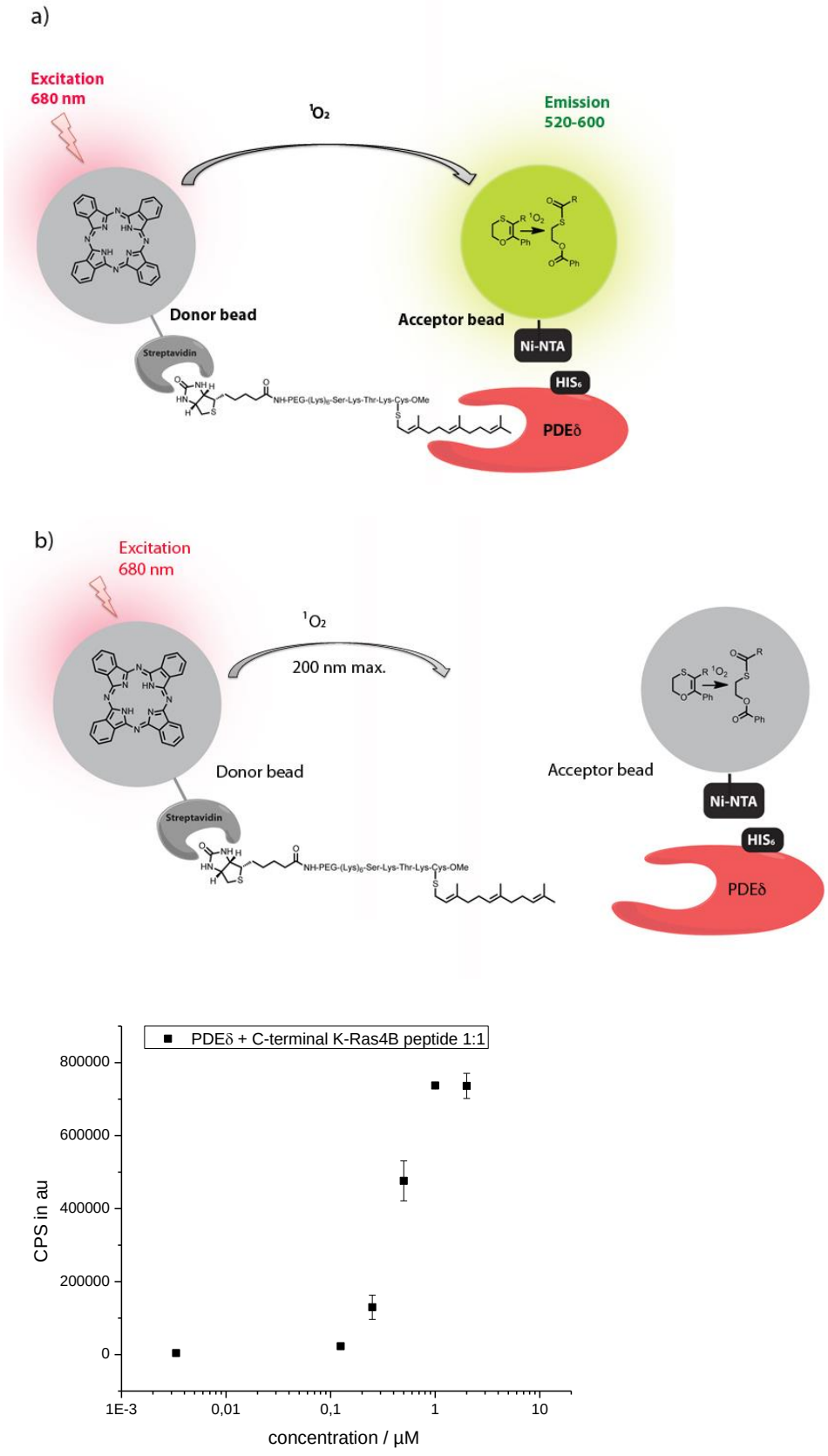
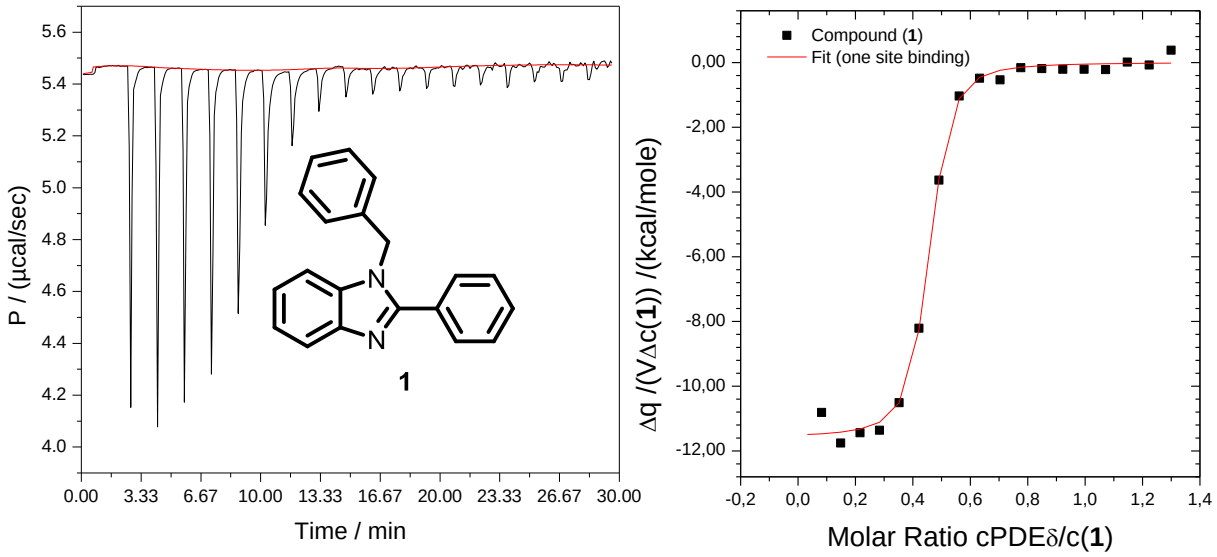
doi:10.1038/nature12205



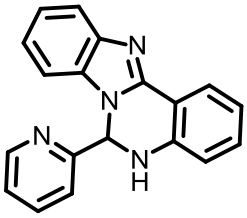
Supplementary Figure 1. Design of a high-throughput screening assay for the detection of small molecule inhibitors of the K-Ras4B/PDEδ interaction. a) The assay is based on the chemiluminiscence-based Alpha Screen technology and relies on a donor bead that recognizes

the biotinylated peptide and an acceptor bead that binds to the His-tagged protein. b) In the presence of an inhibitor no emission between 520-600 nm is observed. Lower panel: Initial development of a screening assay by a serial dilution of a 1:1 complex of PDEδ with a C-terminal K-Ras4B peptide. Chemiluminescence was detected as described below (cps: counts per second, au: arbitrary units). Error bars in the graph are derived from a triplicate determination.

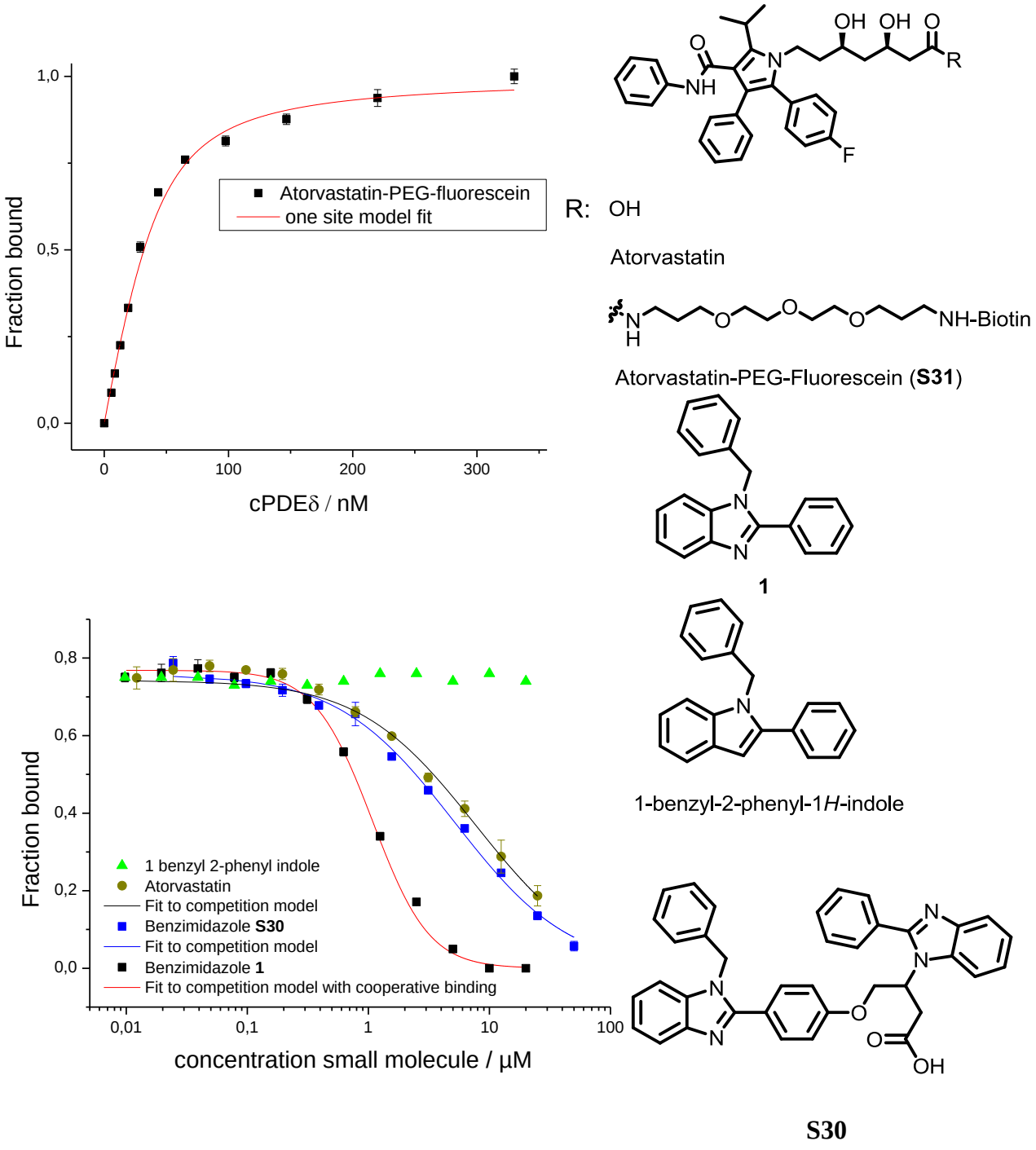


Supplementary Figure 2. Detection of the binding of benzimidazole **1** to PDEδ by isothermal titration calorimetry (ITC). Protein was titrated to compound as described below. Fitting to a one-site model yields a K_D of 217±15 nM. P: power; Δq /VΔc: heat released per mole of injectant.

For the related, racemic benzimdazole **S1** a binding affinity of 1.42 ± 0.48 µM was detected by ITC.

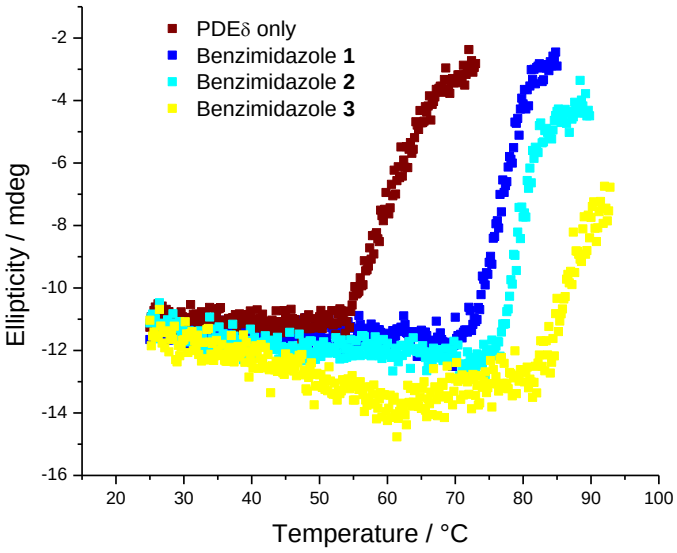


rac-**S1**

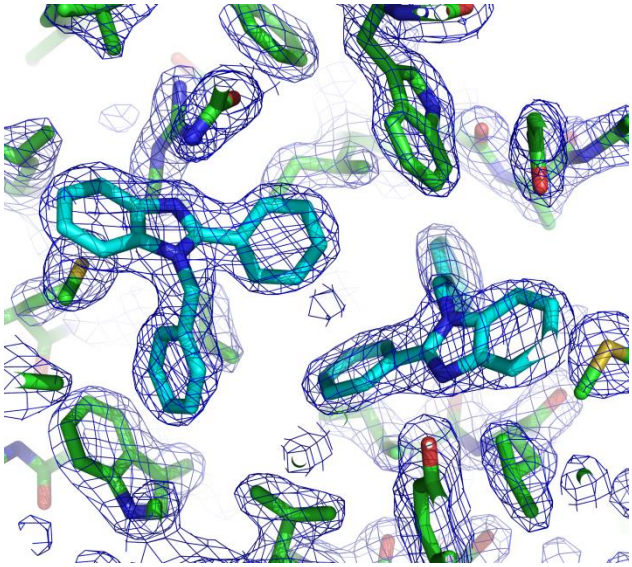


Supplementary Figure 3. Upper Panel: Direct titration of Atorvastatin-PEG-Fluorescein (**S31**) (50 nM) with PDEδ. Fluorescence anisotropy data were converted into fraction bound of Fluorescein-labeled probe and fitted to a one-site binding model. Lower Panel: Representative displacement titrations of Atorvastatin-PEG-Fluorescein with small molecules **1**, **S30**, the related N-benzyl indole and Atorvastatin, and fit to competition models. Conditions: (Atorvastatin PEG-Fluorescein 24 nM, PDEδ 40 nM). Data for Benzimidazole **1** were fitted to a numeric simulation of a competition binding model that takes into account the cooperative binding of two molecules **1**; Data for **S30** and Atorvastatin were fitted with an

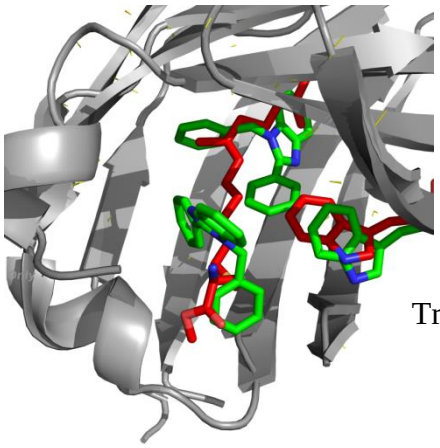
exact mathematical solution to a competition model derived from the law of mass action for details see below. (K_D values from at least three independent titrations: **1** 165 ± 23 nM, **S30,** 870 ± 290 nM, Atorvastatin 1,250 ± 234 nM, Atorvastatin PEG-Fluorescein 7.1 ±4 nM).



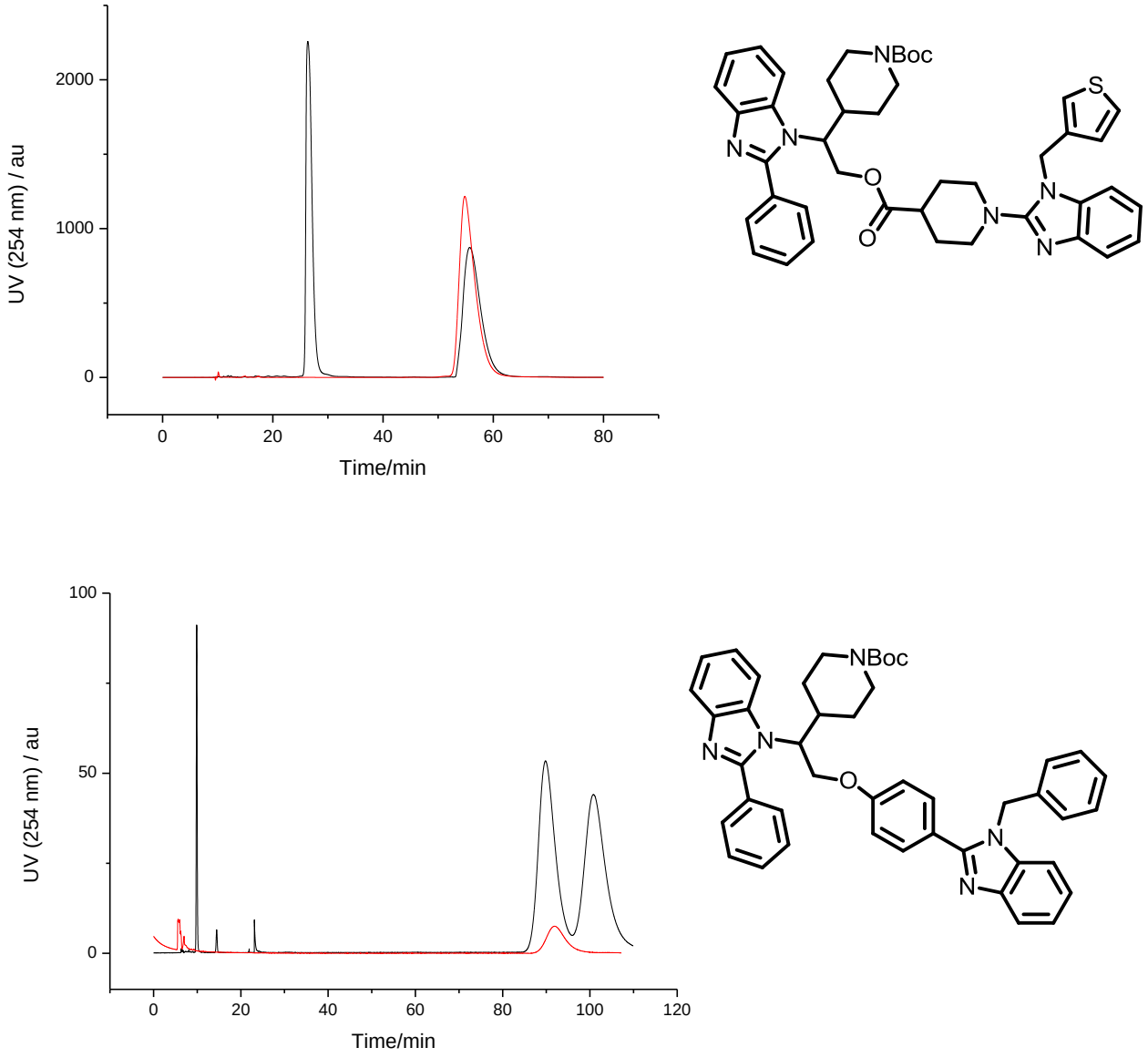
Supplementary Figure 4. Representative plots for the determination of the change in protein melting point (T_m shift) of PDEδ by addition of small molecule (20 μM PDEδ, 30 μM small molecule; for compound **1,** 50 μM compound was used). CD-spectroscopy was employed to measure the unfolding of protein at 233 nm as described below.



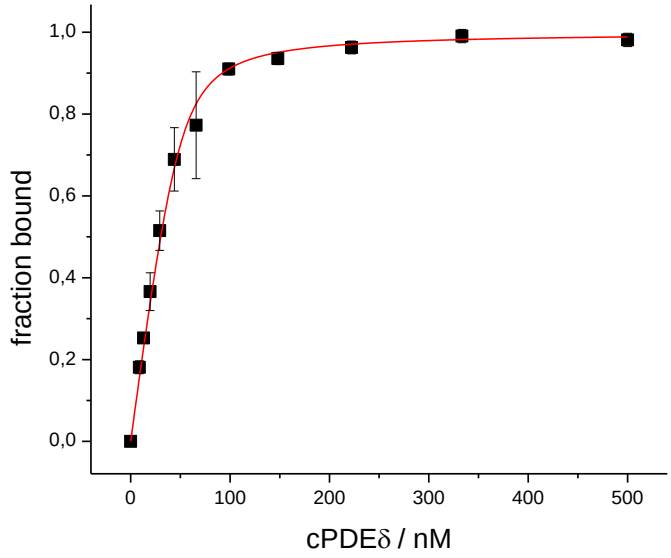
Supplementary Figure 5. Electron density map of **1** in the complex structure with PDEδ.

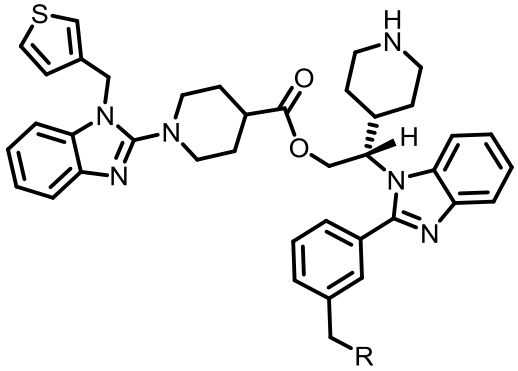


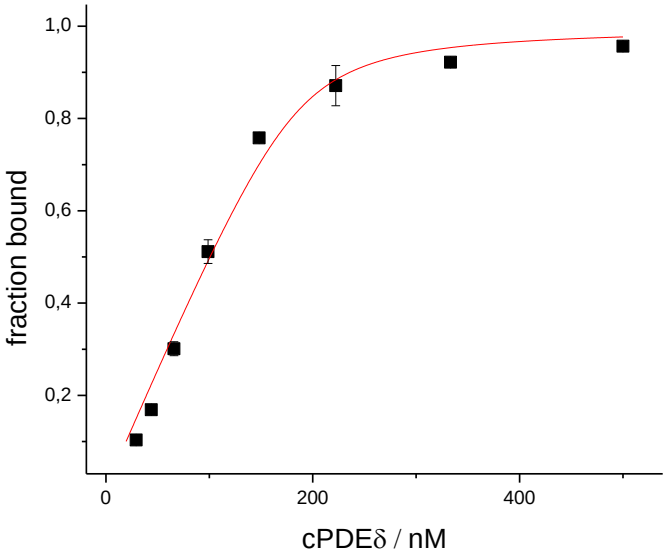
Supplementary Figure 6. Overlay of the farnesyl group (red, from Rheb-PDEδ structure, PDB 3T5G) with structure of **1** and PDEδ. The flip of Trp90 opens the hydrophobic tunnel and results in a T-stacking interaction of Trp90 with one of the phenyl rings of benzimidazole **1**.

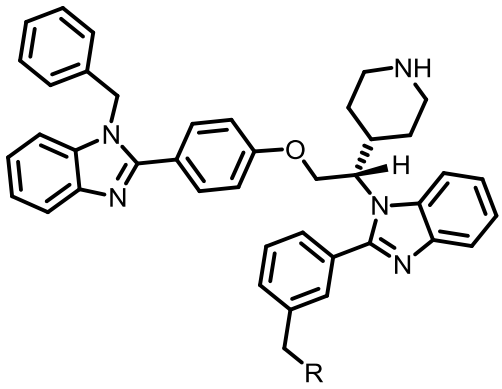


Supplementary Figure 7. Chiral HPLC traces for racemic benzimidazoles Boc-**6** (upper panel,black) and Boc-**4** (lower panel,black) and for the (*S*)-enantiomers (red) obtained by both preparative chiral HPLC and enantioselective synthesis. Sterochemistry was assigned according to literature precedents.[1]

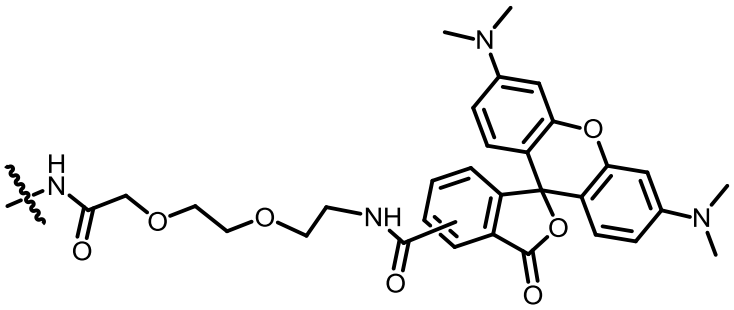




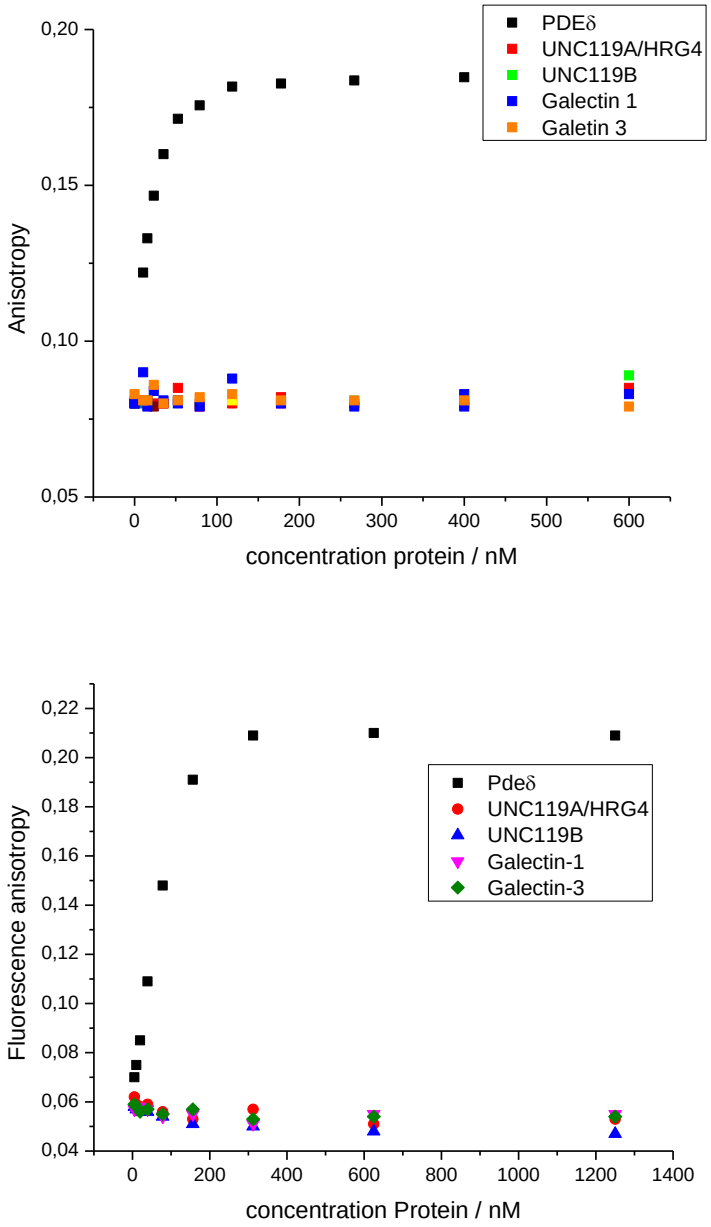




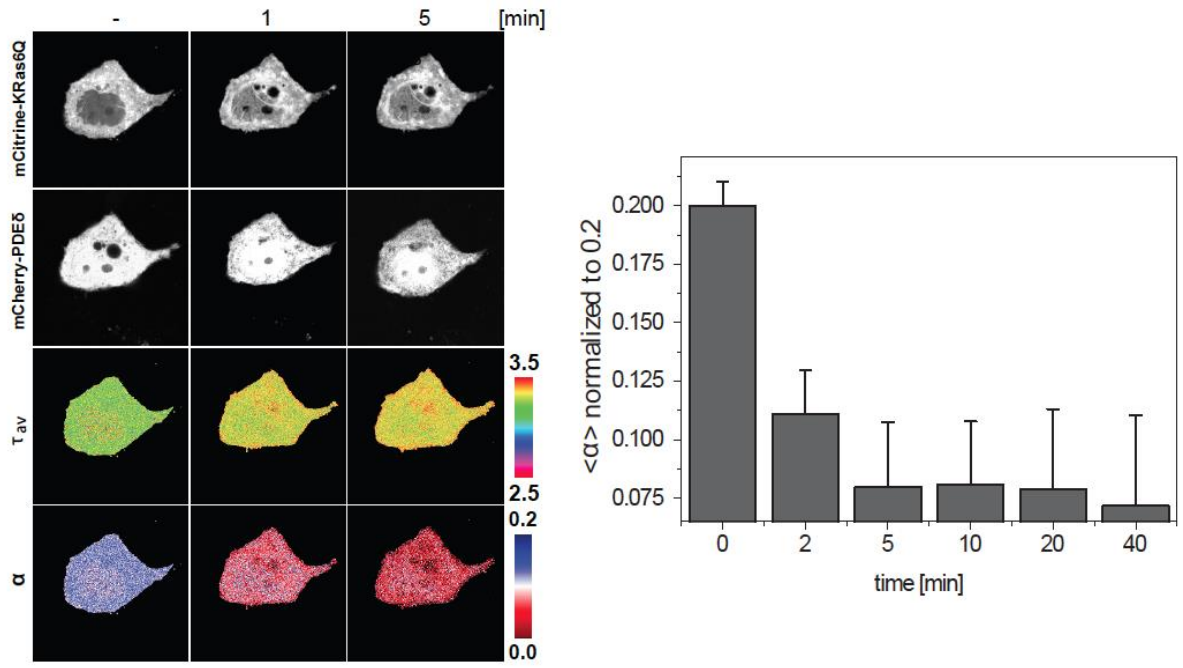
R:



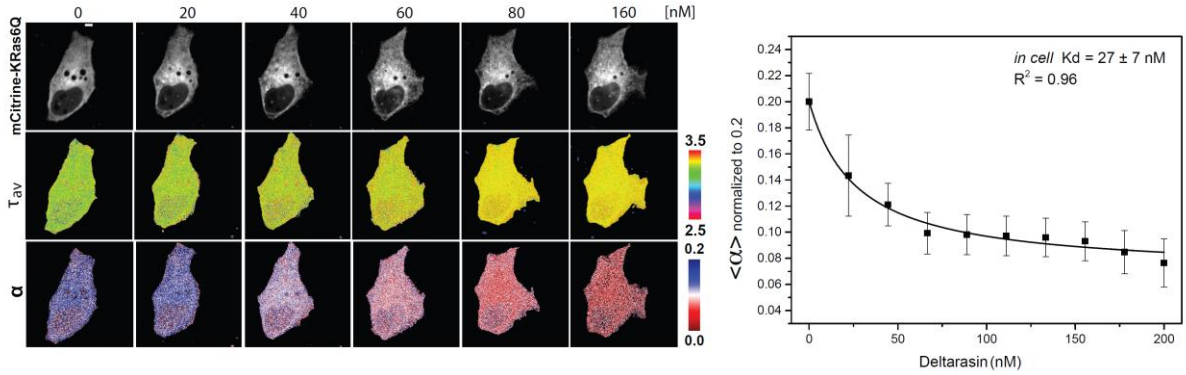
Supplementary Figure 8. Direct Titration of TAMRA-(*S*)-**6** (100 nM, upper panel) and TAMRA-(*S*)-**4** (175 nM, lower panel) with PDEδ. Fit to a one-site binding model derived from the law of mass action yields the corresponding K_D values. (TAMRA-(*S*)-**4** K_D 7.6 ± 1.3 nM and TAMRA-(*S*)-**6** (5.3 ± 1.5 nM).



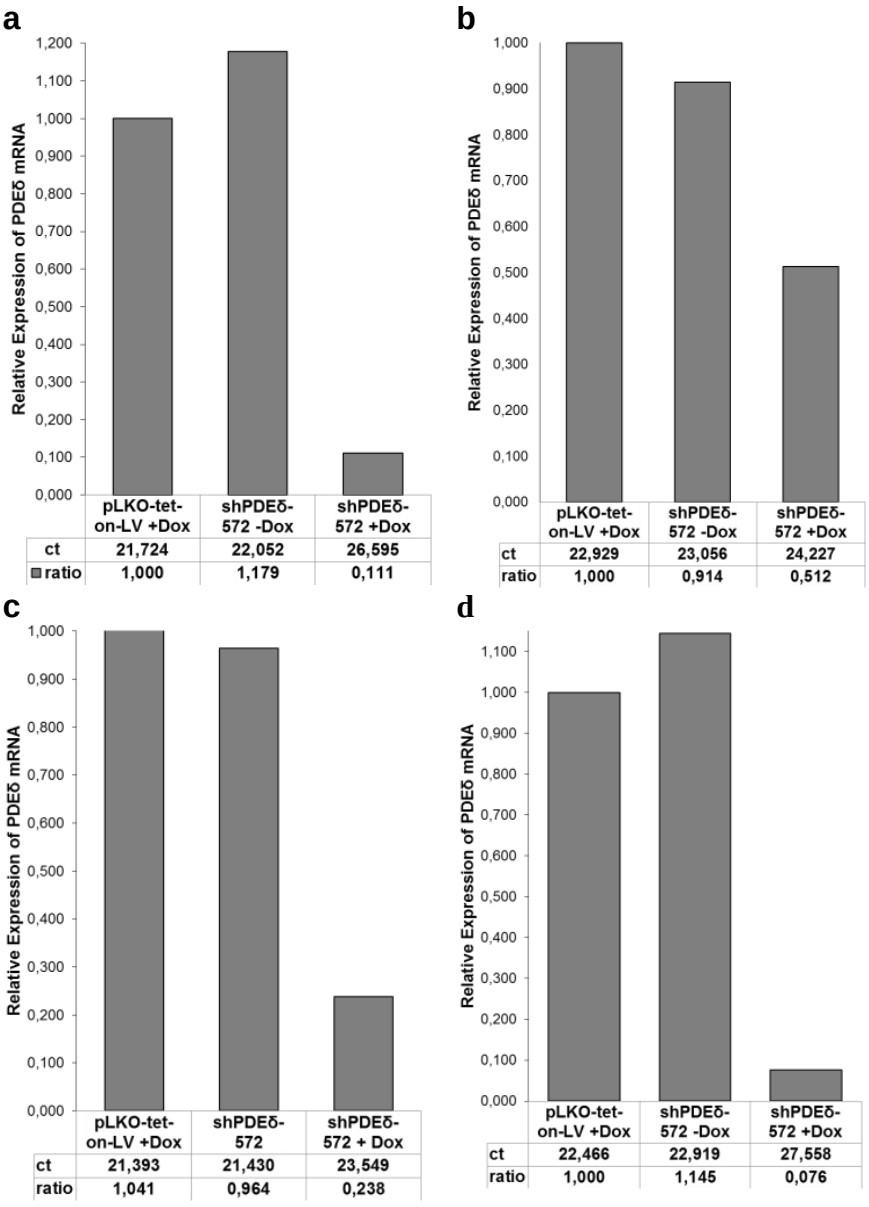
Supplementary Figure 9. Direct titration of TAMRA-(*S*)-**6** (100 nM, upper panel) and TAMRA-(*S*)-**4** (175 nM, lower panel) with PDEδ, UNC119A/HRG4, UNC119B, Galectin-1 and Galectin-3 in PBS buffer (containing 0.05% Chaps) and measured changes of fluorescence anisotropy. Titrations for all proteins other than PDEδ were repeated with 1 µM TAMRA-(*S*)-**6**, however no binding isotherm was observed for any of the mentioned proteins.



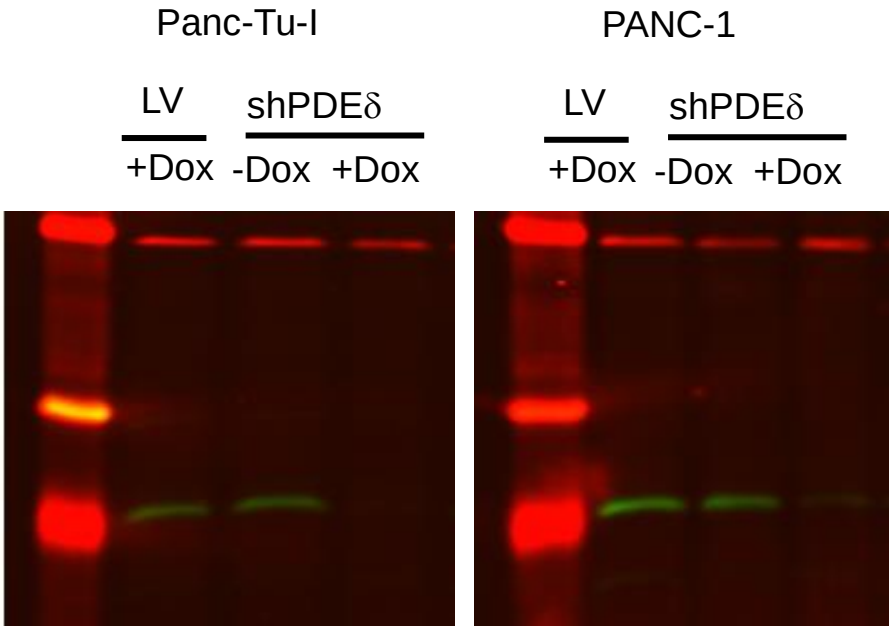
Supplementary Figure 10. FLIM time series on MDCK cells expressing mCitrine–Kras6Q and mCherry–PDEδ show a loss of interaction between Kras6Q and PDEδ after treatment with 2 µM Deltarasin ((*S*)-**4**). Left panel: representative sample of FLIM time series. Upper two rows show fluorescence intensity distribution of the indicated fluorescent fusion proteins, while the lower two rows show maps of average fluorescence lifetime (τ_{av}) in ns and computed molar fraction (α) of interacting mCitrine-Kras6Q with mCherry-PDEδ. Time in minutes is indicated above the panels. The Deltarasin induced dissociation of mCherry-PDEδ and mCitrine-Kras6Q is represented in the time course of normalized average α for N=4 cells in the right panel.



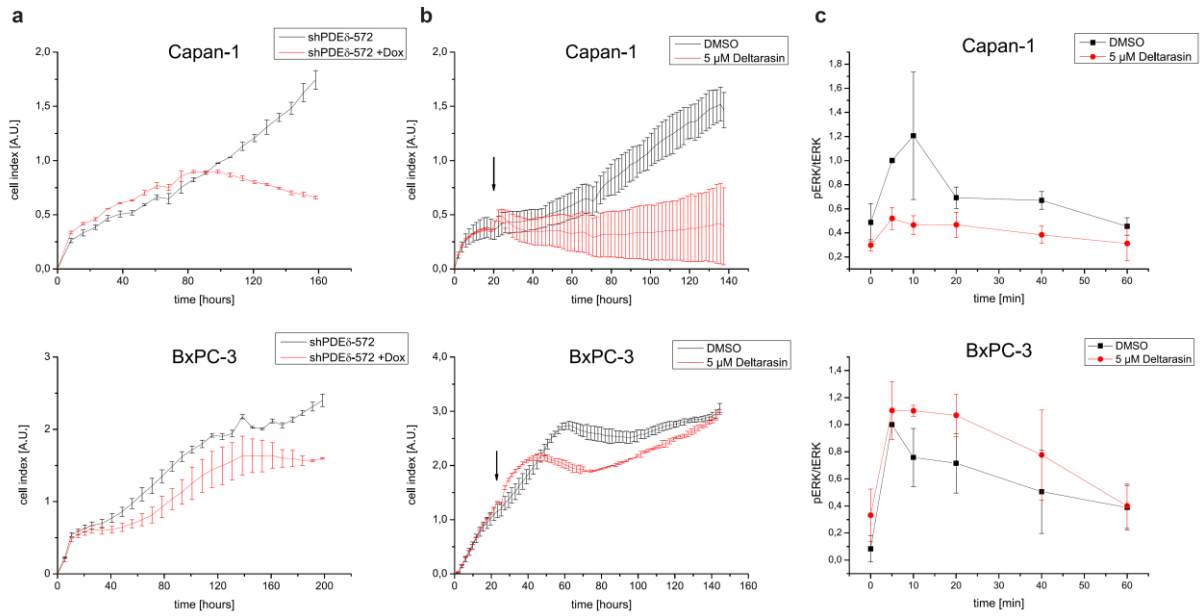
Supplementary Figure 11. Left panel: Deltarasin dose dependence of molar fraction (α) of interacting mCitrine-Kras6Q with mCherry-PDEδ. Upper row shows fluorescence intensity distribution of mCitrine-Kras6Q, middle row shows average fluorescence lifetime (τ_{av}) in ns and lower row shows molar fraction (α) of interacting mCitrine-Kras6Q with mCherry-PDEδ. The concentration of Deltarasin ((*S*)-**4**) is indicated at the top of the panel in nM. Right panel: fit of averaged dose-response of four independent experiments to a binding model (see methods) yielded an in cell K_D of 27 +/- 7 nM for Deltarasin binding to PDEδ.



Supplementary Figure 12. Confirmation of the inducible PDEδ knock-down at the transcript level. qRT-PCR results showing the expected inducible down-regulation of PDEδ at the transcript level in the four cell lines **a,** Panc-Tu-I, **b,** BxPC3, **c,** PANC-1 and **d,** Capan-1. Shown are expression levels relative to the PDEδ expression in the empty vector control. GAPDH and PPIA were used for normalization. *Ct*, raw cycle threshold level.

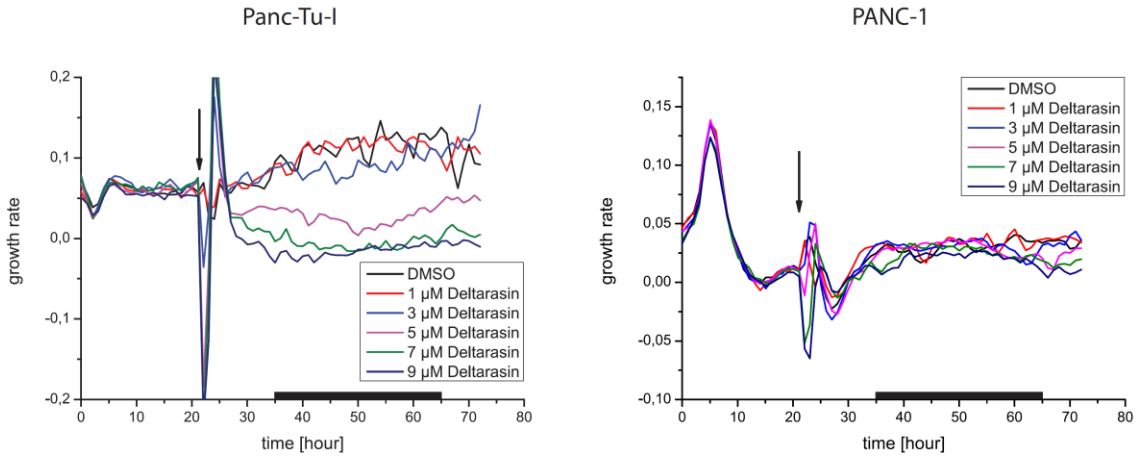


Supplementary Figure 13. PDEδ protein expression following shRNA knock-down. Shown are two representative Western blot examples showing down-regulation of PDEδ protein levels after 4 days of doxycycline induction of the shRNA-PDEδ transgene expression. GAPDH shown as red bands is a loading control (approx. 36 kD). PDEδ protein is shown as green bands (approx. 16 kD). *LV*, empty shRNA pLKO-Tet-ON vector, *shPDEδ,* shRNA vector pLKO-Tet-ON-PDE6D-572, *+Dox,* cells treated with doxycyclin, *-Dox,* no doxycycline treatment.

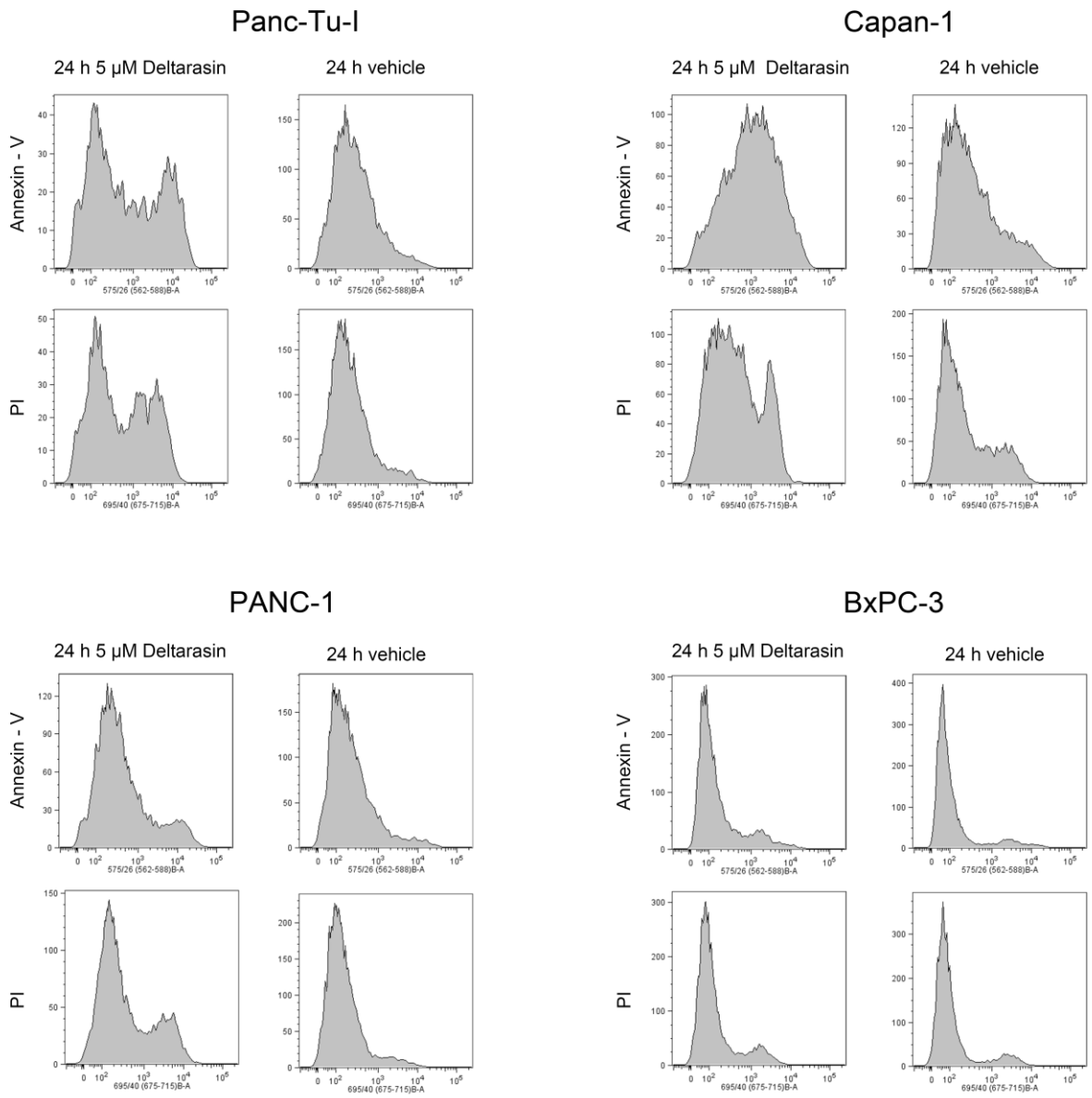


Supplementary Figure 14. Knock-down of PDEδ and Deltarasin ((*S*)-4) suppress Kras dependent hPDAC proliferation and Deltarasin ((*S*)-4) impairs EGF mediated MAPK-signaling in Kras dependent cells.

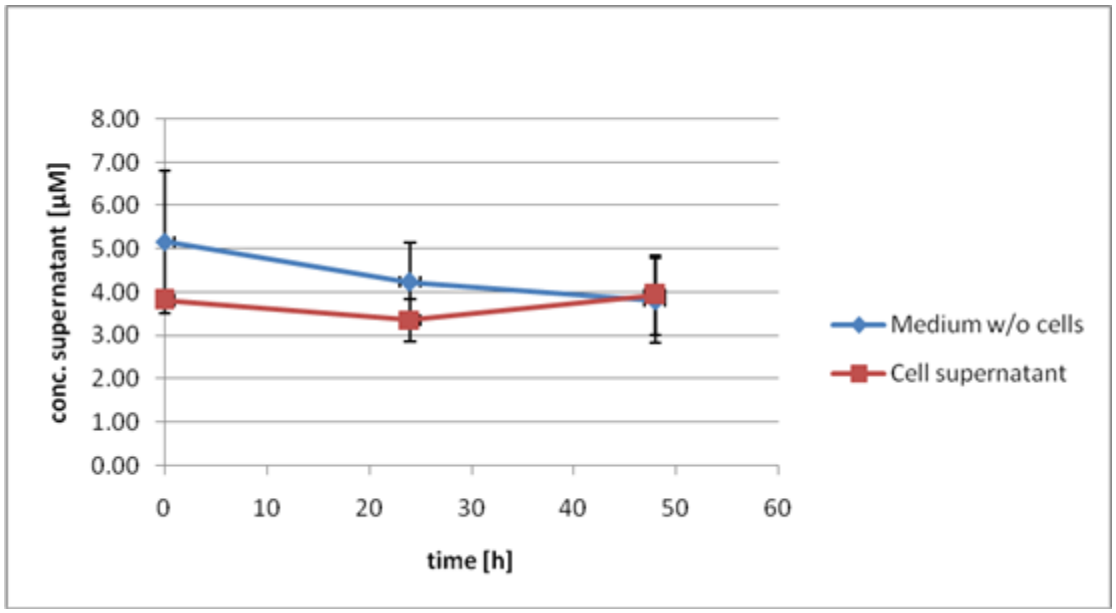
a, Real time cell analysis (RTCA) of oncogenic Kras dependent Capan-I (upper panel) and wild-type Kras expressing BxPC-3 (lower panel) hPDAC cell proliferation after doxycycline induced PDEδ knock-down. Cells were transduced with shPDEδ-572 doxycycline-inducible knock-down vector. Cell indices were measured in duplicates. Cells were treated with doxycycline to induce PDEδ knock-down from the beginning of the experiment (+ Dox). b, Real time cell analysis (RTCA) of 5 µM Deltarasin dose hPDAC cell proliferation response of oncogenic Kras dependent (Capan-I, upper panel) and independent (BxPC-3, lower panel) cell lines shows Deltarasin induced suppression of proliferation in oncogenic Kras dependent cell lines. Deltarasin was added at the indicated time point (arrow). c, EGF-induced MAPK signaling response in hPDAC cells treated with Deltarasin. Peak normalized Erk1/2 phosphorylation time profiles upon stimulation with 200 ng/ml EGF in serum starved Capan-I and BxPC-3 cells as quantified from three independent Western blots for each cell line. Each Western blot contained the Erk1/2 time response with and without Deltarasin (Supp. Fig. 17). The average +/- SD is shown for each time point. Black: vehicle control DMSO, red: 2 h incubation with 5 µM Deltarasin prior to EGF administration.



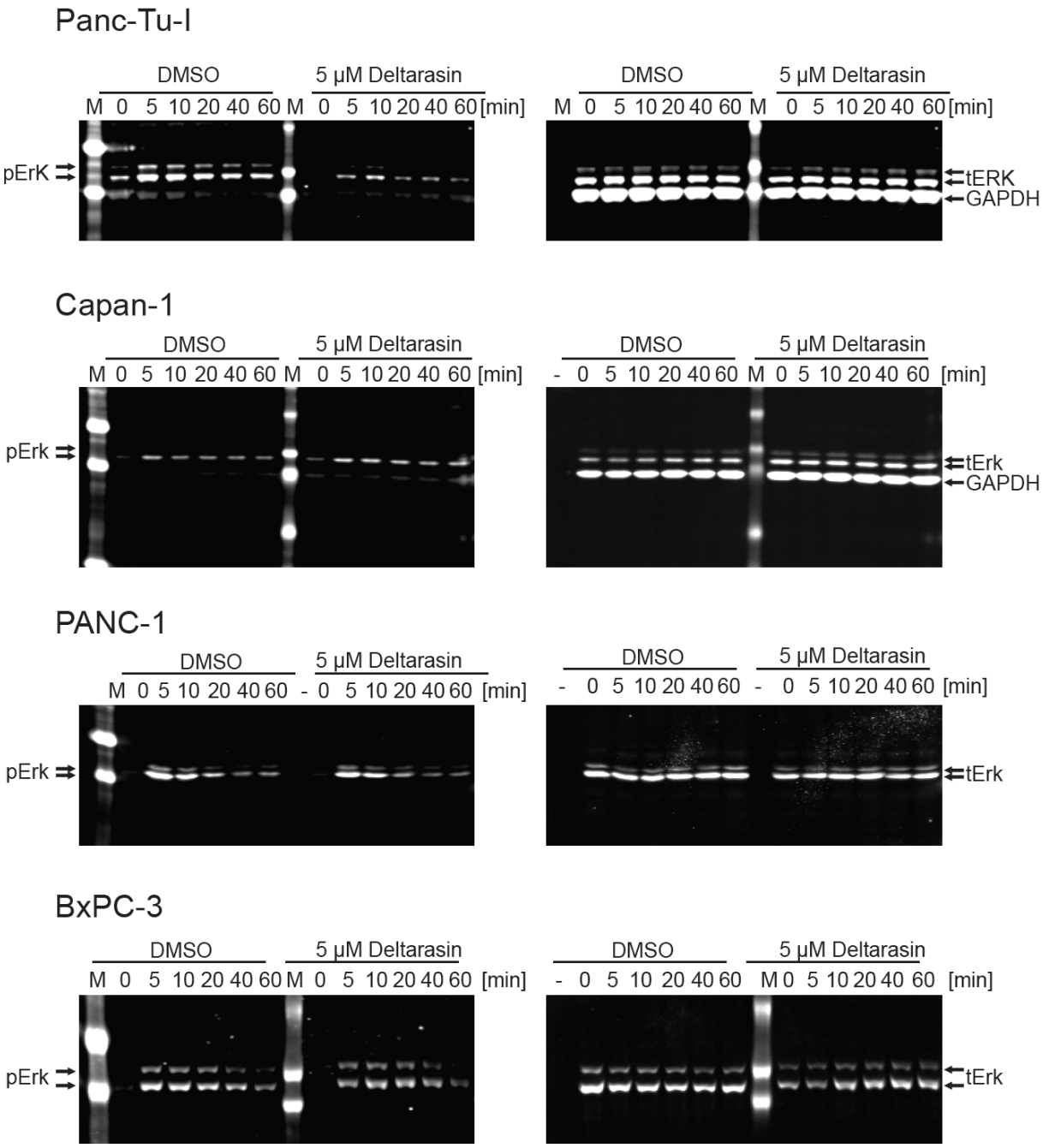
Supplementary Figure 15. Growth rate (first derivative) derived from Deltarasin ((*S*)-**4**) dose hPDAC cell proliferation response of oncogenic Kras dependent (Panc-Tu-I, left panel) and independent (PANC-1, right panel) cell lines (Figure 3b). The average growth rate for each concentration (plotted in Figure 3b) was calculated for the time points between 35 and 65 (indicated by the black bar at the abscissa). Time point of Deltarasin administration is indicated by an arrow.



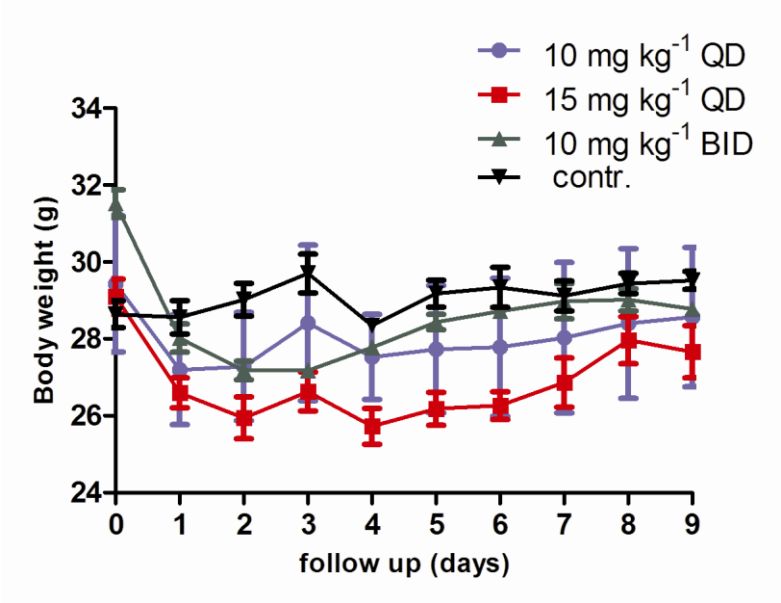
Supplementary Figure 16. Deltarasin (*(S)*-**4**) induced cell death in Kras dependent hPDAC cells (Capan-1 and Panc-Tu-I) as measured by an Annexin-V-PE/propidium iodide FACS analysis. Kras dependent Panc-Tu-I, Capan-1 and Kras independent PANC-1 and BxPC-3 hPDAC cells were measured after 24 hours of vehicle DMSO and 5 µM Deltarasin incubation. The histograms in the upper row of each panel represent the fluorescence intensity profile of the Annexin-V-PE staining (Apoptosis-marker); the histograms in the lower row present the fluorescence intensity profile of propidium iodide (PI) staining. The labels at the abscissa indicate the spectral selection by filters used for fluorescence measurements in the format: median transmission/bandwidth (spectral transmission range).



Supplementary Figure 17. Determination of the stability of Deltarasin ((*S*)-**4**) in the presence of Panc-Tu-I cells. Panc-Tu-I cells were seeded in 2 ml complete growth medium (s.a.) at a density of 200000 cells per 6 well (Greiner). Deltarasin was added to wells in duplicates at 5 µM final concentration. After 0, 24 and 48 hours of incubation at 37°C/5% CO_2 samples were taken and analyzed for Deltarasin concentration by means of HPLC-LCMS. Compound incubated with medium under same conditions served as a control.

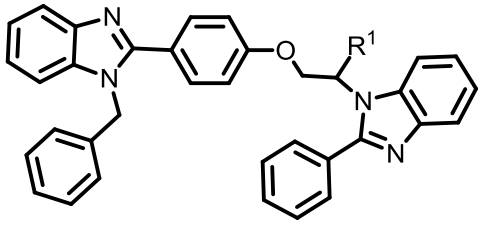


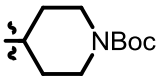
Supplementary Figure 18. Western blots of an EGF stimulus dependent Erk1/2 phosphorylation time profile in serum starved hPDAC cells. Each row presents one representative Western blot for the indicated cell line. Each Western blot contained the total and phosphorylated Erk1/2 time response (time indicated above the blots) with DMSO and 2 hours incubated Deltarasin ((*S*)-**4**) (indicated above the blots).



Supplementary Figure 19. Body weight distribution of mice during Deltarasin ((*S*)-**4**) treatment**.** Body weights of mice in each treatment group are shown as mean values. Error bars represent s.e.m. with n=5 for the control, 10 mg kg^{-1}BID, and 15 mg kg^{-1}QD and n=6 for the 10 mg kg^{-1} QD group.

Supplementary Table 1. Structure-activity-relationships for ether-linked benzimidazoles with different substituents at R^1. K_D values were determined employing the fluorescence polarization-based assay.



Entry	R^1	K_D / nM	T_m shift /
1	CH_2COOH	870 ± 290	10.0
2	H	116 ± 29	17.6
3		>2000	7.2

Full methods

Biochemical Experiments

Alpha-Screen:

Screening based on Alpha-technology was conducted in white, non-binding 1536-well plates (Corning) in a final volume of 6 µL. For the screen a mixture of His_6-PDEδ, and biotinylated K-Ras-peptide (final concentrations 100 nM and 250 nM in HEPES 20 mM, 100 mM NaCl, 0,005% Chaps, pH 7.5) were added to the 1536-well plates. Compound solutions were directly added from 10 mM DMSO stock solutions to a final concentration of 10 µM and the resulting mixture was incubated for 30 min (For dose-response curves, compounds were tested at concentrations betwen 10 µM and 5 nM). Premixed Nickel Chelate Acceptor Beads and Streptavidin Donor Beads were added to a final concentration of 10 µg/mL. The resulting mixture was incubated at 4°C overnight. Plates were read on a Paradigm reader (Molecular devices, Alphascreen 1536 HTS detection cartridge, temperature 29°C-33°C).

Fluorescence polarization measurements for the determination of the binding constant K_D of TAMRA-labeled (*S*)-4 and (*S*)-6

PDEδ was serially diluted in a solution containing of TAMRA-labeled (*S*)-**4** or (*S*)-**6** in 200 µL PBS-buffer (containing 0.05% Chaps, 0.5% DMSO). The 96-well plates were incubated at room temperature for 3 h. The fluorescence polarization values (Ex: 530 nM, Em: 580) were read on a Safire II plate reader (Tecan, temperature ~30°C).

Data was corrected for changes in fluorescence intensity and the fraction bound was fitted to a one-site binding model derived from the law of mass action using K_D as the only fitting parameter.[2] Non-linear regression was performed in OriginPro 8.6.

$$FSB = \frac{A - A_{free}}{A - A_{free} + Q(A_{bound} - A)}$$

FSB=fraction bound; A_{free}: Anisotropy of free fluorophore A_{bound}: anisotropy of bound fluorophore; Q: change in fluorescence intensity between free and bound state; A: observed Anisotropy.

$$FSB = \frac{K_D + L_{Tot} + P - \sqrt{(K_D + L_{Tot} + P)^2 - 4L_{Tot}P}}{2L_{Tot}}$$

$L_{Tot:}$ total concentration of fluorophore, P: protein concentration

Data for Atorvastatin-PEG-Fluorescein were fitted analogously.

Displacement titrations of labeled Atorvastatin-probe for the determination of K_D values:

Binding to PDEδ was validated and quantified by means of a displacement assay employing a fluorescent-tagged analog of the HMG-CoA reductase inhibitor Atorvastatin (Lipitor®) which has previously been shown to also bind to PDEδ.[3] For the displacement assay, a fluorescent-tagged analog of Atorvastatin bearing a polyethylenglycol linker and a fluorescein moiety was synthesized (see below, **S31**) Atorvastatin K_D = 1,250 ± 234 nM, Atorvastatin PEG-Fluorescein K_D = 7.1 ± 4 nM).

To fluorescein-labeled Atorvastatin **S31** (24 nM) and His_6-tagged PDEδ (40 nM) in 200 µL PBS-buffer (containing 0.05% Chaps, 1% DMSO) in a black, non-binding 96-well plate (Greiner) was added the small molecule from a DMSO stock solution. The resulting solution was serially diluted, holding the concentrations of fluorescein-Atorvastatin, PDEδ and DMSO constant. The sealed plates were shaken overnight at room temperature and centrifuged at the beginning of the next day. The fluorescence polarization values (Ex: 470 nm, Em: 525 nm) were read on a Safire II plate reader (Tecan, temperature 28-32°C).

For the fitting of the data to a competition model the exact mathematical solution derived from the law of mass action was used as described by Roehrl *et al.*[4] Fraction bound data was fit using KD2 as the only fitting parameter and total ligand concentration as independent variable (OriginPro8.6, function codec from origin).

FSB=(2*(sqrt((KD1+KD2+LST+LT-RT)^2-3*((LT-RT)*KD1+(LST-RT)*KD2+KD1*KD2)))*cos((acos((-2*(KD1+KD2+LST+LT-RT)^3+9*(KD1+KD2+LST+LT-RT)*((LT-RT)*KD1+(LST-RT)*KD2+KD1*KD2)-27*(-KD1*KD2*RT))/(2*sqrt(((KD1+KD2+LST+LT-RT)^2-3*((LT-RT)*KD1+(LST-RT)*KD2+KD1*KD2))^3)))/3)-(KD1+KD2+LST+LT-RT))/(3*KD1+2*sqrt((KD1+KD2+LST+LT-RT)^2-3*((LT-RT)*KD1+(LST-RT)*KD2+KD1*KD2))*cos((acos((-2*(KD1+KD2+LST+LT-RT)^3+9*(KD1+KD2+LST+LT-RT)*((LT-RT)*KD1+(LST-RT)*KD2+KD1*KD2)-27*(-KD1*KD2*RT))/(2*sqrt(((KD1+KD2+LST+LT-RT)^2-3*((LT-RT)*KD1+(LST-RT)*KD2+KD1*KD2))^3)))/3)-(KD1+KD2+LST+LT-RT)).

FSB: Fraction bound of fluorescent probe

KD1: Binding constant of fluorescent Probe determined from direct titration

KD2: Fitting parameter (Binding constant of unlabeld competitor)

LST: Total concentration of labeled small molecule

LT: Total concentration of unlabeled small molecule competitor

RT: Total protein concentration

For the fitting of data to a competition model with cooperative binding of two benzimidazole fragments the program Scientist 3.0 was used. An experimental system was defined as a series of partial equations including the equilibrium relationships between the various species. Anisotropy data was converted to fraction bound and fitting was carried out in anlogy to a previously described set of equilibrium relationships by Goody and coworkers.[5]

$$K_{D1} = \frac{[A][PDED_f]}{[APDED]}$$

$$K_{D2} = \frac{[L]^2[PDED_f]}{[LPDED]}$$

A: equilibrium conentration of free fluorophore

L: equilibrium concentration of free ligand

APDED: equilibrium concentration of bound fluorescent probe

LPDED: equilibrium conentration of bound small molecule

PDEDf: equlibrium conentration of free protein

This model was used (together with equations describing the mass balance) to numerically simulate the FSB of fluorophore and compared with the experimental data.

Scientist input file:

// Micromath Scientist Model File

IndVars: LST

DepVars: A,L,APDED,LPDED,PDEDf,FSB

Params: ATOT,PDED,KD1,KD2

LPDED= L^2*PDEDf/KD2

APDED=A*PDEDf/KD1

ATOT=A+APDED

LST=L+LPDED

PDED=PDEDf+APDED+LPDED

0<A<ATOT

0<L<LST

0<PDEDf<PDED

FSB=1-A/ATOT

LST=0

Isothermal titration calorimetry

Titrations were carried out on an iTC_{200} calorimeter (MicroCal Inc). PDEδ protein (280 µM) was titrated to small molecule (30 µM) in 50 mM Tris, pH 7.5, 150 mM NaCl containing a final amount of 5% DMSO. Titrations were carried out at 25°C and data were fitted by using a single-site binding model as implemented in the software of the instrument supplier.

T_m-Shift assays

Protein melting points in the presence of small molecules were detected by CD-spectroscopy (Jasco J-815, 0.1 cm path length cell). Melting curves were run in PBS-buffer (1% DMSO) containing 20 µM of small molecule and 30 µM PDEδ. Due to absorption of DMSO unfolding of PDEδ was detected at 233 nm (heating rate 1°C per minute, starting from 30°C).

Crystallography:

Inhibitors **1** and *rac*-**S1** were co-crystallized with PDEδ by mixing a solution of 850 µM small molecule and 400 µM PDEδ with 1.7% DMSO as a final concentration. Crystals were obtained from a Qiagen classic suite (100mM Sodium acetate pH 4.6 2.0 M NaCl). Crystals for *rac*-**2** were obtained similarly, however here a solution of 400 µM inhibitor was mixed with an equimolar solution of PDEδ. Crystals for (*S*)-**5** were obtained in a condition containing 18% PEG 4000, 25mM Sodium Acetate and 200 mM Ammonium Sulphate. The crystals were flash frozen in a buffer that contained the mother liquor components in addition to glycerol as a cryoprotectant. Data collection was done at the X10SA beamline of the Suisse Light Source, Villigen. Data were processed by XDS. Molecular replacement was done using the program MolRep and PDEδ, without bound farnesyl group, from the complex PDEδ-farnesylated Rheb complex was used as a search model (PDB 3T5G) for each data set. Refinement of the models was done using a combination of manual refinement using the program COOT and the maximum likelihood restrained refinement with the program REFMAC5. Ramachandran plot statistics showed none of the residues to be outliers (see data collection and refinement tables).

Supplementary Table 2. Data collection and refinement statistics.

	1	*rac*-***S1***
Data collection		
Space group	P 3_2 21	P 3_2 21
Cell dimensions		
a, *b*, *c* (Å)	55.36,55.36, 115.56	55.97,55.97, 115.56
α, β, γ (°)	90, 90, 120	90,101.33,90
Resolution (Å)	1.87	1.45
R_{sym} or R_{merge}	7.8 (60.5)	5.6(50.3)
I / σI	17.36 (3.72)	24.25(4.7)
Completeness (%)	99.4 (99.1)	99.9 (99.6)
Redundancy	9.7 (10.2)	9.69 (9.5)
Refinement		
Resolution (Å)	1.87	1.45
No. reflections	16641	36030
R_{work} / R_{free}	17.6/22.9	17.9/20.75
No. atoms		
Protein	1205	1182
Ligand/ion	44	46
Water	158	261
B-factors		
Protein	33.1	19.7
Ligand/ion	37.1	18.5
Water	44.8	37.6
R.m.s. deviations		
Bond lengths (Å)	0.023	0.034
Bond angles (°)	2.051	2.68

Values in parentheses correspond to the high resolution shell

Supplementary Table 3. Data collection and refinement statistics.

	rac-**2**	(*S*)-**5**
Data collection		
Space group	P 3_2 2 1	P 3_2 2 1
Cell dimensions		
a, *b*, *c* (Å)	55.68, 55.68, 116.55	55.91, 55.91, 116.67
α, β, γ (°)	90, 90, 120	90, 90, 120
Resolution (Å)	1.75	2.4
R_{sym} or R_{merge}	5.1 (56.3)	14.1 (62.7)
I / σI	27.55 (4.85)	14.05(3.1)
Completeness (%)	99.8 (99.5)	99.0 (98.8)
Redundancy	9.5 (9.2)	5.01(5.3)
Refinement		

Resolution (Å)	1.75	2.4
No. reflections	20677	8225
R_{work} / R_{free}	18.45/23.38	20.69/25.65
No. atoms		
Protein	1188	1158
Ligand/ion	43	48
Water	173	47
B-factors		
Protein	30.5	31.3
Ligand/ion	39.5	26.2
Water	42.8	32.8
R.m.s. deviations		
Bond lengths (Å)	0.029	0.021
Bond angles (°)	2.68	1.98

Values in parentheses correspond to the high resolution shell

Determination of compound solubility and cell permeability

Kinetic solubility was determined by a direct UV assay.[6] Cell permeability of compounds was determined by a parallel artificial membrane permeability assay (PAMPA).[7] Flux [%] denotes concentration (test well)/concentration (control well)_100. Flux values above 25% generally denote high cell permeability.(For Deltarasin a flux of 44.2 % was determined).

Cell Biology

Cell lines and culture conditions. With the exception of Panc-Tu-I (kind gift from H. Kalthoff), all cell lines were purchased from ATCC. For cell lines where reference STR data are available (PANC-1, BxPC-3 and Capan-1, HEK293T), the GenomeLab Human STR Primer Set (Beckman Coulter, Krefeld, Germany) was used and analyzed on a CEQ8800 sequencer (Beckman Coulter) according to the manufacturer's protocol. STR data were submitted to an online verification tool of DSMZ to confirm the identity of these human cell lines (http://old.dsmz.de/human_and_animal_cell_lines/main.php?contentleft_id=101).
All cell lines were cultured in DMEM supplemented with 10% fetal calf serum, 100 U/ml penicillin and 100 µg/ml streptomycin (Invitrogen-Gibco, Karlsruhe, Germany). Cells were maintained in a humidified incubator with 10% CO_2 at 37°C. Selection with puromycine was done for all cell lines at a concentration between 0.4-0.6 µg/ml. Induction of the shRNA transgene expression was done with 0.2 µg/ml doxycycline. All cells were routinely maintained in DMEM supplemented with 10% FBS, 1% non-essential amino acids and 1% L-glutamine. For EGF-induced Erk response analysis, hPDAC cells were plated at a density of 4 × 10^5 cells/well in 6-well plates (Falcon). Cells were starved for 16h prior to incubation for 2h with 5 µM of *(S)*-**4**. 200 ng/ml EGF was applied to the cells for the indicated time and washed with ice-cold PBS and 0.1 ml ice-cold lysis buffer containing protease inhibitors (Roche) and phosphatase inhibitor I and II (Sigma). Cells were further processed as under 'Western blots'. For live-cell microscopy, cells were cultured 4-well Lab-Tek chambers (NUNC) and transferred to low-bicarbonate DMEM without phenol red supplemented with 25 mM HEPES at pH 7.4. Transfections were carried out with Effectene (Qiagen) and Lipofectamine 2000 (Invitrogen) for hPDAC cells.

Western blots. Cells were scraped off after 5 min on ice and centrifuged at 14,000g for 20 min at 4° C. SDS–PAGE was carried out with 30 µg of whole-cell lysate from each sample. Cell lysates for treated and control cells at six time points after EGF stimulation were loaded onto the same gel to allow direct comparison by image processing. The gels were blotted onto PVDF membrane (Millipore) and blocked for 1 h at room temperature. Antibodies used for western blotting were: total Erk (Abcam-AB36991; 1:2,000), pErk (Cell Signaling-9101; 1:2,000), GAPDH (Calbiochem-CB1001; 1:5,000) and infrared secondary antibodies (LI-COR). Blots were scanned on a LI-COR Odyssey imaging system. Infrared signals from pErk

and total Erk were detected in separate fluorescence channels. The relative fraction of phosphorylated Erk (pErk/Erk) for each condition was determined by dividing the fluorescence images of the blot in the two channels after background subtraction. Average pErk/Erk pixel values were determined for each Erk band corresponding to an experimental condition (time of EGF stimulation, +/- (*S*)-**4**).

RNA-Isolation

Total RNA was isolated from cells using the standard acid guanidinium thiocyanate-phenolchloroform extraction procedure (2). Cells were lysed in solution D (4 M guanidium thiocyanate, 0.1 M ß-mercaptoethanol, 0.5% sodium N-laurylsarcosine, 25 mM sodium citrate, pH 7.0), 0.1 volume 2 M sodium acetate pH 4.0, 1 volume water-saturated phenol, and 0.2 volume chloroform:isoamyl alcohol (25:1) were added. After centrifugation, the aqueous phase was removed and RNA was precipitated with the addition of 2 volumes of ethanol. RNA pellets were resuspended in solution D and precipitated a second time by the addition of 2 volumes ethanol. Following centrifugation, RNA pellets were resuspended in RNase-free dH_2O. RNA quantity and quality was assessed by measuring the optical density at 260 and 280 nm and by standard RNA agarose gel electrophoresis.

Quantitative real-time Polymerase Chain Reaction (qRT-PCR)

First-strand cDNA was synthesized with Moloney Murine Leukemia Virus *Reverse Transcriptase* (200 U/µl, *Promega, Mannheim, Germany*) using 1 µg total RNA. Exon-intron border spanning and gene-specific primers were obtained using the Primer Express (V.2.0, Applied Biosystems, Foster City, USA). Primer sequences were: *PDE6D*, forward primer 5'-TCCGCCTGGAACAAAAAGTT-3' and reverse primer 5'- TCGGGTGCTGCCTCTATCA-3'. qRT-PCR experiments were carried out using SYBR Green (Cambrex Corp., East Rutherford, NJ) at a final volume of 20 µL with the cycle protocol recommended by the manufacturer. Samples were run on an Engine Opticon 2 system with a PTC-200 DNA Engine Cycler and a CFD-3220 Opticon 2 detector (MJ Research, Waltham, MA). Reactions were carried out using primers at a final concentration of 0.2 µM and cDNA as a template. PCR cycling conditions were composed of an initial step at 94°C for 3 min followed by 40 cycles of 94°C for 30 s, 60°C for 30 s, 72°C for 30s, and a step of 82°C for 5 s. Fluorescence was measured at the last step of each cycle. Melting curves were obtained after each PCR run and showed single PCR products. All cDNA samples, non-RT (without reverse transcriptase) and no-template controls were assayed in triplicate. The housekeeping genes *GAPDH* and

PPIA were used to normalize the expression data according to Pfaffl et al.[8] and LinRegPCR program version 12.0.

Vectors

pLKO-shRNA-PDE6D vectors. For selecting the most effective RNA targeting site and excluding of-target effects three *pLKO-shRNA-PDE6D vectors* targeting different regions of PDE6D were constructed. All three vectors consistently knocked-down PDE6D transcripts and gave a consistent growth inhibition phenotype in MIA PaCa-2. The vector-targeting region 572 being most effective was selected for subsequent experiments. Vectors were constructed by annealing the *sense* and *antisense* oligonucleotides to each other and subsequent cloning of the resulting double stranded DNA molecule into the *AgeI/EcoRI* site of the *pLKO-puro* vector (kindly provided by Sheila Stewart). Oligonucleotides for cloning were:

pLKO-shRNA-PDE6D-402:

sense: 5´-CCGGGCAATGCCTAGAAGAATGGTTCTCGAGAACCATTCTTCTAGG CATTGCTTTTTG-3', *antisense*: 5´- AATTCAAAAAGCAATGCCTAGAAGAATGGTT CTCGAGAACCATTCTTCTAGGCATTGC-3´

pLKO-shRNA-PDE6D-500:

sense: 5´-CCGGTGATGCCAGCAAGCGTCTTAACTCGAGTTAAGACGCTTGCTGG CATCATTTTTG-3', *antisense*:5´-AATTCAAAAATGATGCCAGCAAGCGTCTTAA TCGAGTTAAGACGCTTGCTGGCATCA-3'

pLKO-shRNA-PDE6D-572:

sense: 5´- CCGGGCACATCCAGAGTGAGACTTTCTCGAGAAAGTCTCACTCTGG ATGTGCTTTTTG-3', *antisense*: 5´- AATTCAAAAAGCACATCCAGAGTGAGACTTT CTCGAGAAAGTCTCACTCTGGATGTGC-3'

***pLKO-Tet-ON-shRNA-PDE6D-572* vector.** The vector *Tet-pLKO-puro* was purchased from addgene (Plasmid 21915) and the H1-promoter replaced by the U6 promoter to form the vector *pLKO-Tet-ON*. The cloning steps to construct the *pLKO-Tet-ON-shRNA-PDE6D-572* vector were identical with the non-inducible vector described above also including the oligonucleotides used for hairpin formation.

Empty *pLKO-puro* or *pLKO-Tet-ON-puro* vectors were taken as controls. The correct insert sequences of the generated vectors were confirmed by standard cycle sequencing analysis.

Virus production and infection of target cells

Lentiviruses were produced by transfecting packaging cells (HEK293T) with a three plasmid system. For transfections 12 µg pCMVΔR8.2, 6 µg pHIT G and 12 µg plasmid DNA were combined with 62 µl of 2 M $CaCl_2$ in a final volume of 500 µl. Subsequently 500 µl of 2x HBS phosphate buffer was added dropwise and incubated for 10 min at RT. The 1 ml transfection mixture was then added to 50% confluent HEK293T cells into a 10 cm dish. Cells were incubated for 16 h (37°C and 10% CO_2), and the medium changed to remove remaining transfection reagent. 36 h post transfection, lentivirus containing supernatants were collected and filtered (0.45 µm pore size) for subsequent infection of target cells. 10 ml supernatant containing 4 µg /ml polybrene was immediately used to infect target cells in 10 cm dishes at 50% confluency. After incubating the infected cells for 24h, all lentivirus containing supernatants were removed and fresh medium supplied.

Immunostaining of endogeneous Ras

After 2 hours incubation with DMSO, 200 nM and 5 µM Deltarasin, hPDAC cells were washed three times with PBS and fixated with 4% Paraformaldehyde for 5 minutes. After an additional PBS washing step, cells were permeabilized with PBS/0,1%Triton for 5 minutes and three times washed with PBS before 1 hour blocking with blocking buffer (Odyssey 927-40000). The Anti-Pan-Ras mouse monoclonal antibody (Calbiochem OP40-100UG; 1:200) was incubated in blocking buffer for 2 hours and afterwards 3 times washed with PBS/0.1%Tween-20. As secondary antibody an Alexa-647 Donkey-anti-Mouse antibody (Invitrogen; 1:1000) was incubated for 1 hour in blocking buffer. The cells were washed 4 times with PBS/0.1%Tween-20 and stored in PBS for microscopy. All steps were carried out at room temperature.

Image aqusition of the Ras immunostaining

The images were acquired with a confocal laser scanning microscope (SP5, Leica) equipped with an white light laser (WLL). For detection of Alexa-647, the sample was excited using the 647 nm wavelength of the WLL. Fluorescence signal was collected through an oil immersion objective (63x/1.40 HCX PL APO, Leica) and spectrally filtered by a band pass filter from 660 nm to 770 nm.

Fluorescence Lifetime Imaging Microscopy (FLIM). Fluorescence lifetime images were acquired using a confocal laser-scanning microscope (FV1000, Olympus) equipped with a time-correlated single-photon counting module (LSM Upgrade Kit, Picoquant). For detection of mCitrine, the sample was excited using a 470nm diode laser (LDH 470, Picoquant) at a 40 MHz repetition frequency. Fluorescence signal was collected through an oil immersion objective (60x/1.35 UPlanSApo, Olympus) and spectrally filtered using a narrow-band emission filter (HQ 525/15, Chroma). Photons were detected using a single-photon counting avalanche photodiode (PDM Series, MPD) and timed using a single-photon counting module (PicoHarp 300, Picoquant).

FLIM data analysis. Intensity thresholds were applied to segment the cells from the background fluorescence. Data were further analyzed as described in[28] to obtain images of the molar fraction (α) of interacting mCherry-PDEδ/mCitrine-RheB. Dependence of α on Deltarasin concentration was determined through sequential addition of Deltarasin doses followed by incubation for 15 minutes and FLIM data acquisition. Dose-response relationships were determined by plotting the average α per cell for various concentrations of Deltarasin. The *in cell* K_D was estimated from averaged dose-response of 4 independent experiments by fitting to the following function:

$$\alpha/\alpha_0 = C - [I]/([I]+K_d)$$

where α_0 is the molar fraction of interacting mCherry-PDEδ/mCitrine-RheB in the absence of inhibitor, [I] is the concentration of the inhibitor Deltarasin in the medium and C is a constant that represents an asymptotic offset.

FACS analysis. hPDAC cells were seeded at a density of $1{,}5 \times 10^5$ cells/well in 6-well plates. After 24 hours the cells were treated with 5 µM Deltarasin for 24 hours. Medium was transferred to FACS vials, cells were washed two times with PBS and detached by Trypsin/EDTA. All cells were then stained with the PE Annexin V apoptosis detection kit I (BD Pharmingen) according to the manufacture´s protocol. Cells were analyzed on a LSR II flow cytometer (BD Biosciences).

Real time cell analysis. RTCA was performed using 16-well E-plates on the Dual Plate xCELLigence instrument (Roche Applied Science, Indianapolis IN). This system measures a

dimensionless parameter called cell index (CI), which evaluates the ionic environment at an electrode/solution interface and integrates information on cell number. 5000 cells were plated in each well of the 16-well plates in 200 µl of cell culture medium. After seeding, cells were allowed to settle for 30 min at RT before being inserted into the xCELLigence instrument in a humidified incubator at 37°C with 10% CO_2. For RTCA experiments assaying cells stably expressing the inducible shRNA transgene, cells were seeded onto the E-plates in medium supplemented with doxycycline and puromycine. For RTCA experiments assaying cells treated with PDEδ/Ras interaction inhibitor Deltarasin, cells were seeded the day before, Deltarasin was administrated at a concentration of 5 µM. Continuous impedance measurements were then monitored every 15 min up to 200 hours. All assays were performed in duplicates for shRNA experiments and triplicates for the Deltarasin inhibitor.

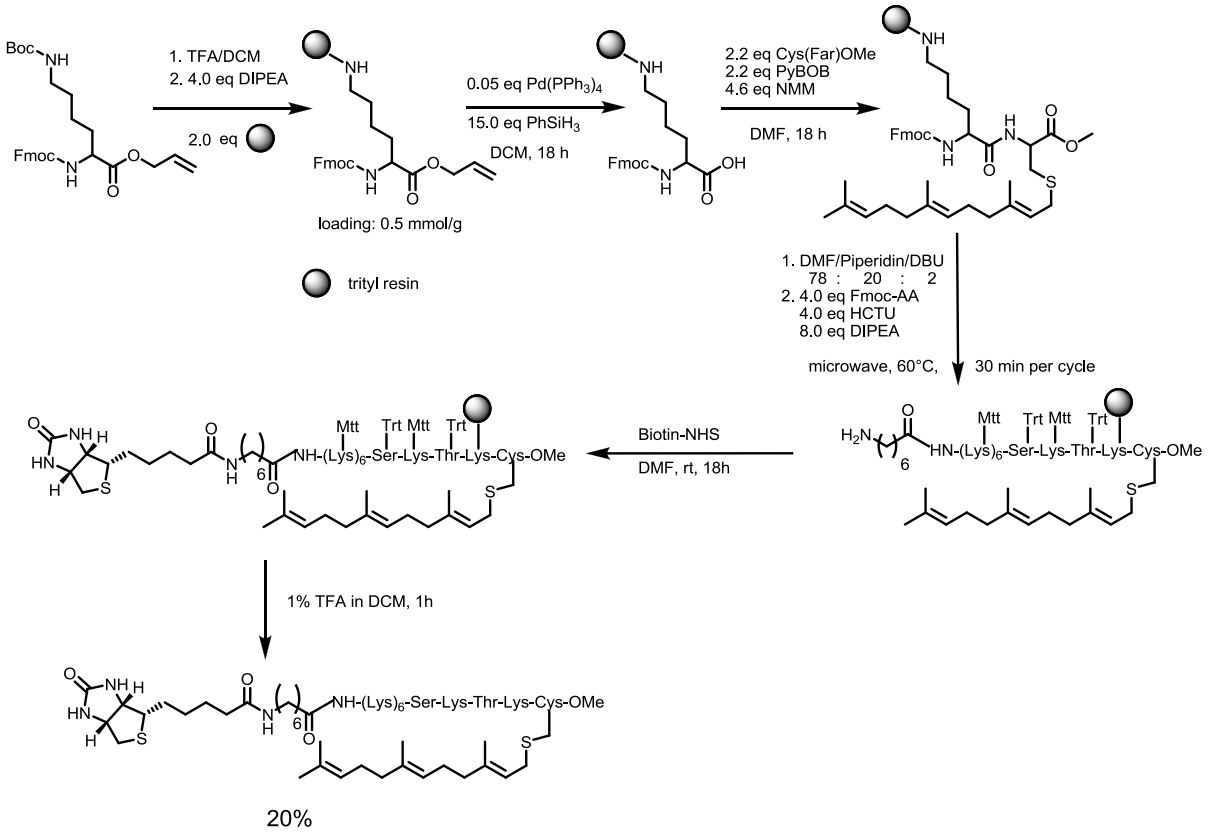
Xenografted tumors. $5x10^6$ Panc-Tu-I cells were implanted into five- to six-week-old female mice (NMRI-*Foxn1nu/Foxn1nu*, Janvier, St Berthevin Cedex, France) by s.c. injection (two injection sites per mouse). Xenografts were allowed to grow to a size around 125 mm^3, then randomized in the following four groups of treatment, with five to six mice in each group: (a) control (Lipovenoes and 5% DMSO); (b) Deltarasin 15 mg kg^{-1} QD i.p.; (c) Deltarasin 10 mg kg^{-1} QD i.p.; (d) Deltarasin 10 mg kg^{-1} BID i.p. PDEδ inhibitor Deltarasin was formulated in Lipovenoes 10% PLR (Fresenius Kabi, Bad Homburg, Germany) and 5% DMSO. Mice were treated during 9 days, weighted daily and monitored for signs of toxicity. Tumor volumes were estimated from two-dimensional tumor measurements every third day by caliper measurements using the following formula: Tumor volume (mm^3) = [length (mm) x width $(mm)^2$]/2. Treatment of mice was stopped once the first tumor reached a volume of 1000 mm^3. Animal experiments and care were in accordance with the guidelines of institutional authorities and approved by local authorities (number: 84-02.04.2012.A328)

Chemical Synthesis

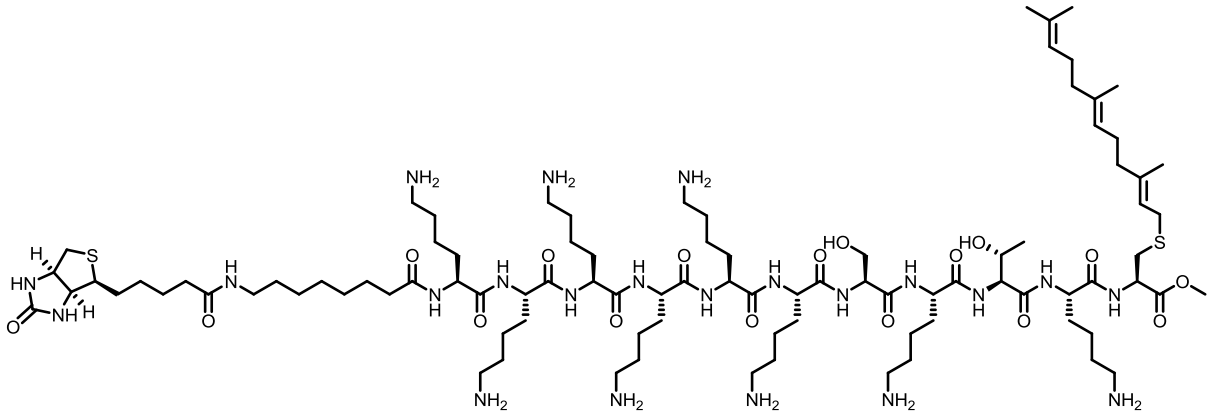
General considerations. NMR spectroscopic data were recorded on a 400 MHz or 600 MHz instrument at room temperature. NMR spectra were calibrated to the solvent signals of deuterated DMSO-d_6 or $CDCl_3$. The following abbreviations are used to indicate the signal multiplicity: s (singlet), d (doublet), t (triplet), q (quartet), br (broad), m (multiplet). Analytical HPLC-MS data were recorded on a HPLC system with a C18 reverse column coupled to an ESI spectrometer, flow rate: 1.0 mL/min; time: 15 min; solvent A: 0.1% HCOOH in water, Solvent B: 0.1% HCOOH in Acetonitrile; 1 min 10% B, in 10 min to 100% B. High resolution mass spectra (HR-MS) were measured using electrospray ionization (ESI). Chiral HPLC analysis was performed on a Chiralpak IC Column 250 mm x 4.6 mm, flow rate: 0.5 mL/min; ethanol in *iso*-hexane. Preparative HPLC separations were carried out using a reversed-phase C18 column (RP C18, flow 20.0 mL/min, solvent A: 0.1% TFA in water, solvent B: 0.1% TFA in Acetonitrile, from 10 % B to 100 % B.

Atorvastatin was purchased from Sequoia Reseach Products, N-Benzyl 2-phenyl indole was purchased from MicroCombiChem. All other commercially available chemicals were purchased from Alfa Aesar, Aldrich, Acros, ABCR and TCI.

Synthesis of biotinylated, C-terminal K-Ras4B peptide



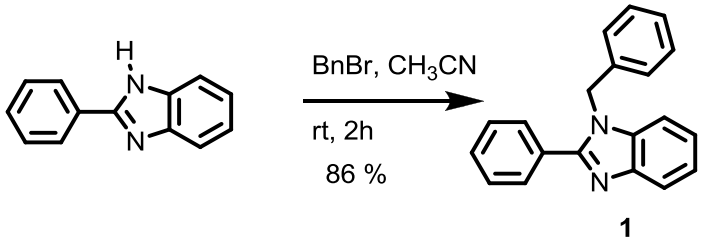
Supplementary Scheme 1. Solid-phase synthesis of a biotinylated *C*-terminal K-Ras4B peptide.



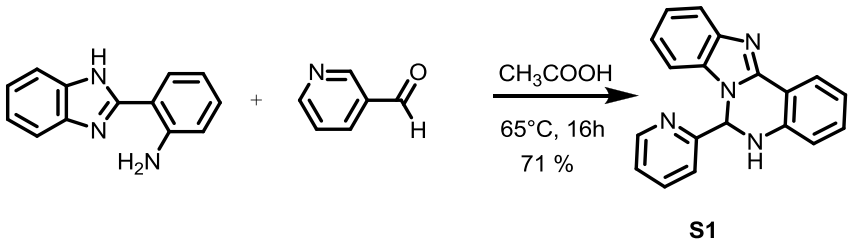
Biotinylated *C*-terminal K-Ras4B peptide was synthesized by means of solid-phase peptide synthesis (scale: 0.1 mmol) analogously to a previously described synthesis.[9] Yield: 40 mg, 0.02 mol, 20%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.51 – 7.68 (m, 31H), 6.42 (s, 1H), 6.38 (s, 1H), 5.19 – 5.01 (m, 3H), 4.52 – 4.10 (m, 11H), 4.01 – 3.94 (m, 1H), 3.66 – 3.53 (m,

5H), 3.22 – 2.94 (m, 4H), 2.90 – 2.59 (m, 18H), 2.19 – 1.85 (m, 8H), 1.77 – 1.14 (m, 64H), 1.02 (d, J = 6.2 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.45, 172.74, 172.59, 172.23, 172.20, 172.15, 171.64, 170.70, 170.20, 163.44, 159.59, 159.39, 159.18, 158.97, 139.40, 135.27, 131.33, 124.76, 124.31, 120.73, 120.68, 118.75, 116.77, 67.33, 62.50, 61.75, 59.91, 58.49, 56.10, 55.59, 53.29, 53.22, 52.97, 52.68, 35.89, 35.81, 32.18, 32.05, 31.95, 31.81, 31.65, 29.84, 29.46, 29.33, 29.16, 28.88, 28.72, 27.29, 27.25, 27.01, 26.85, 26.54, 26.14, 26.04, 25.87, 23.11, 22.90, 22.70, 19.99, 19.91, 18.20, 16.46, 16.41. LC-MS (ESI): calcd for $C_{92}H_{170}N_{22}O_{17}S_2$: 960.63506 $[M+2H]^{2+}$, found 960.97 $[M+2H]^{2+}$, R_t = 4.80 min; HR-MS found 960.63545 $[M+2H]^{2+}$. $[\alpha]_D^{20}$: -66.5° (c=0.72, H_2O).

Synthesis of single benzimidazole fragments:

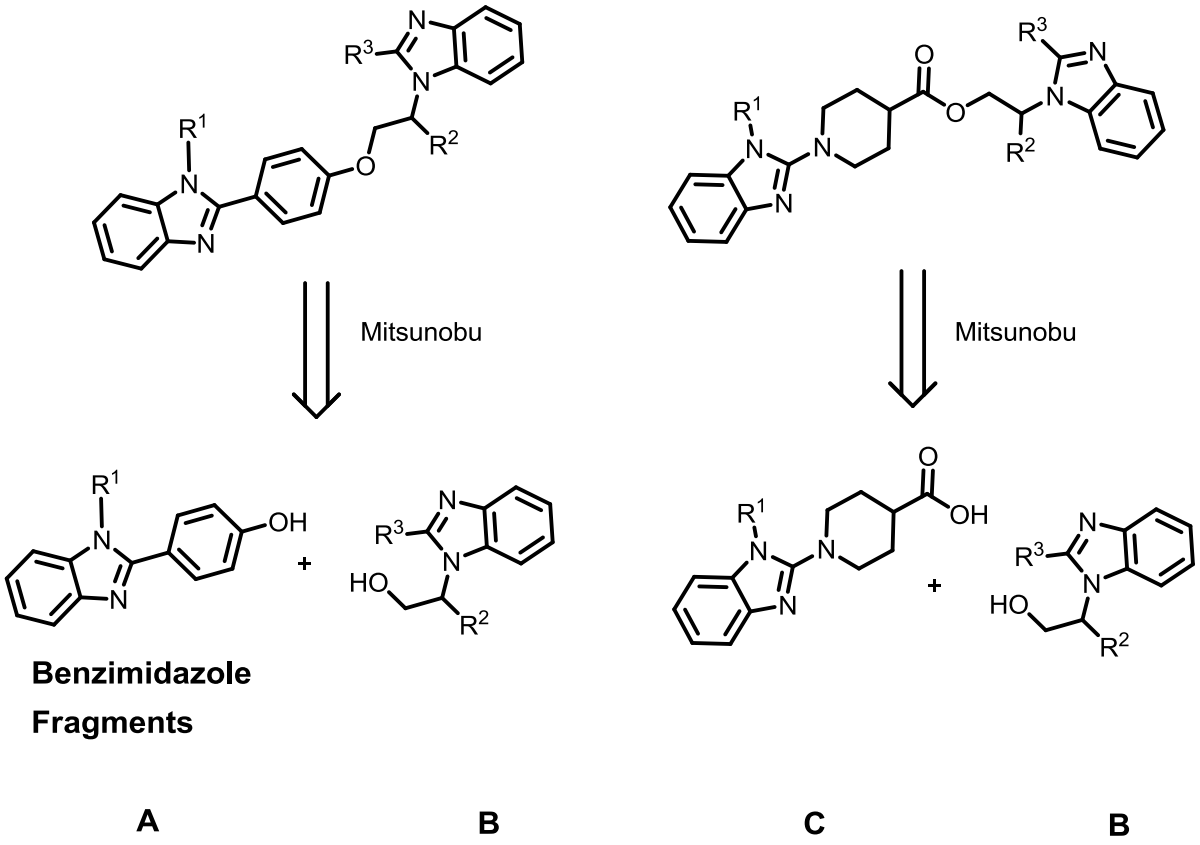


Supplementary Scheme 2. Synthesis of Benzimidazole **1**.



Supplementary Scheme 3. Synthesis of Benzimidazole **S1**.

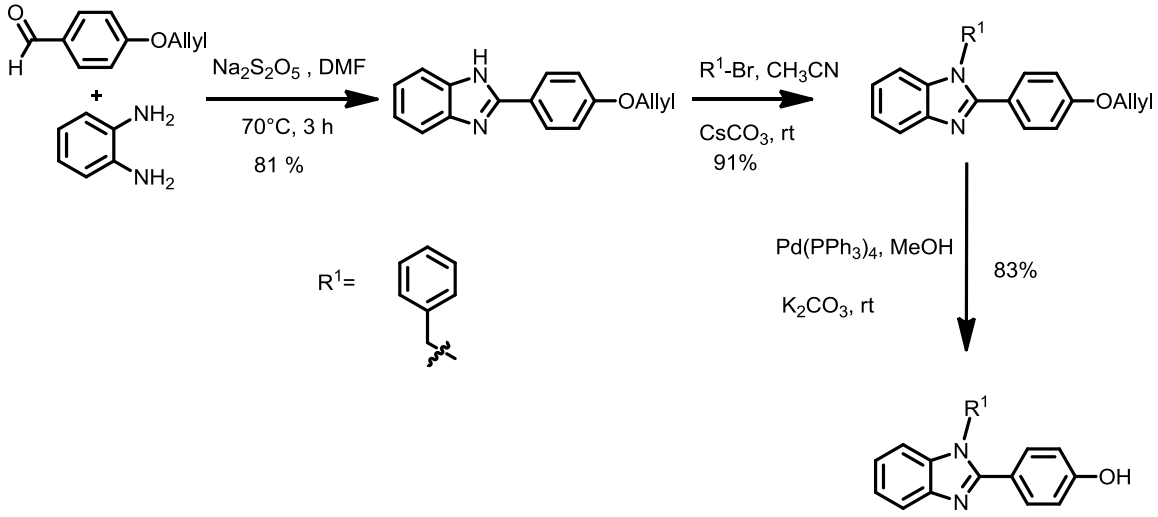
General strategy for the linkage of two benzimidazole units



Supplementary Scheme 4. General strategy for the linkage of two benzimidazole units.

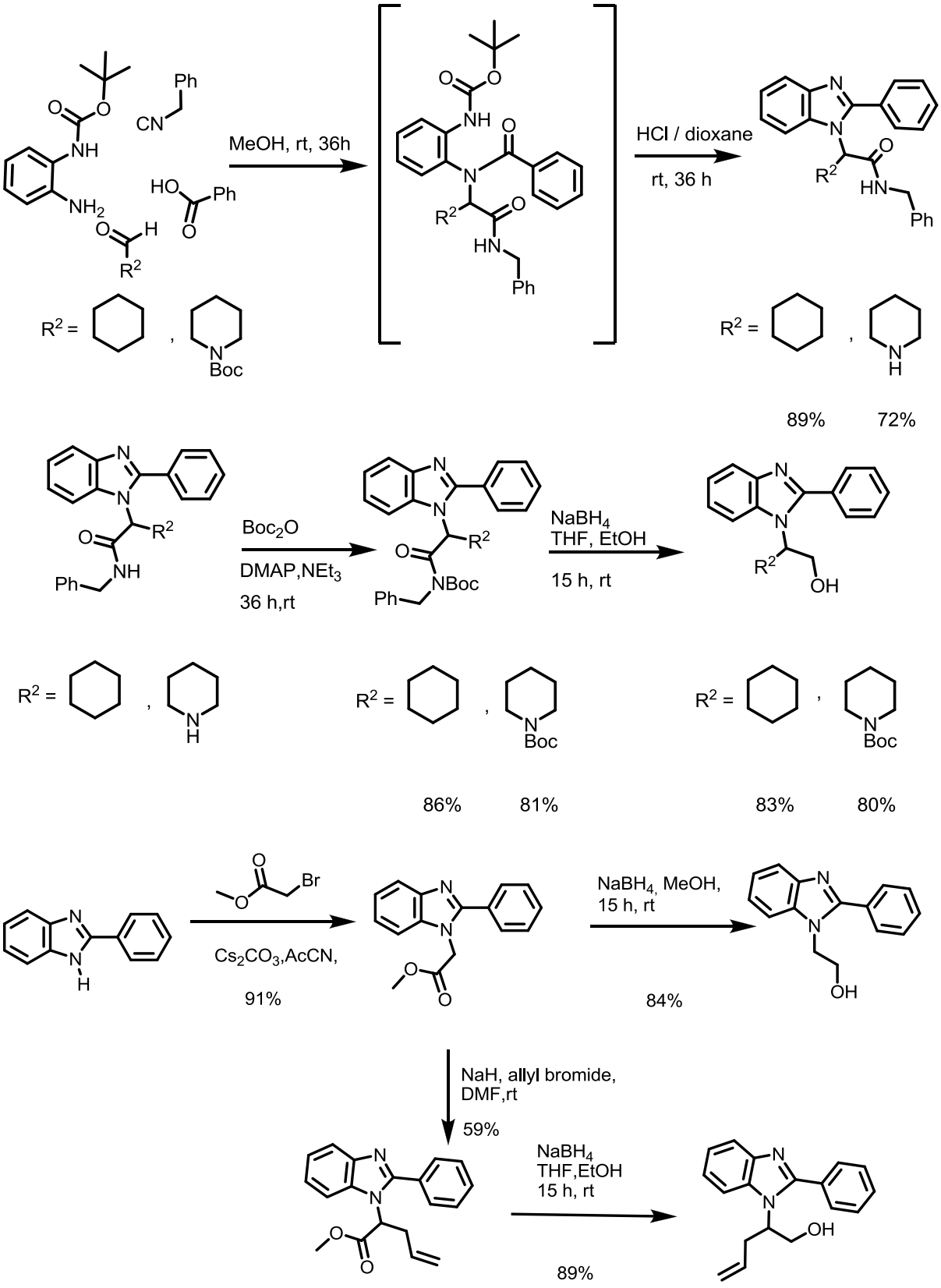
Synthesis of ether-linked benzimidazoles

Synthesis scheme for benzimidazole fragments type A

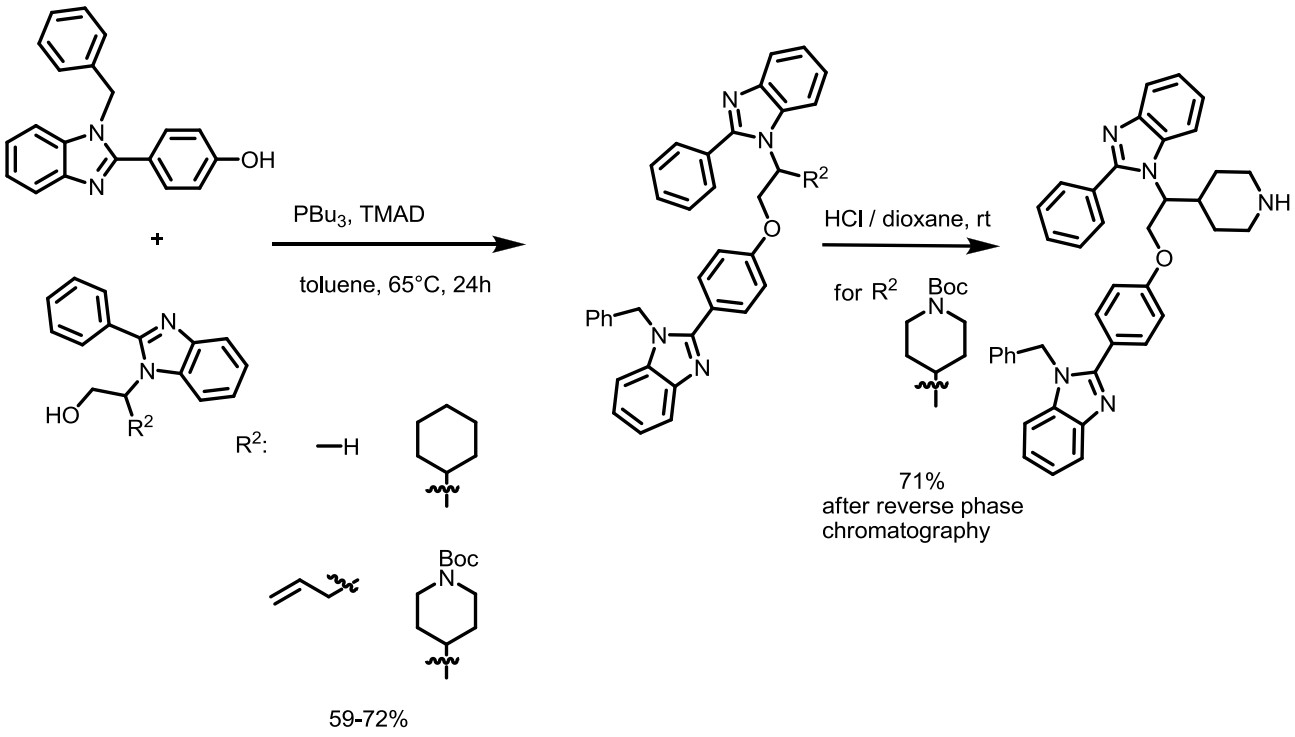


Supplementary scheme 5. Synthesis of benzimidazole-fragments type A.

Synthesis scheme for benzimidazole fragments type B



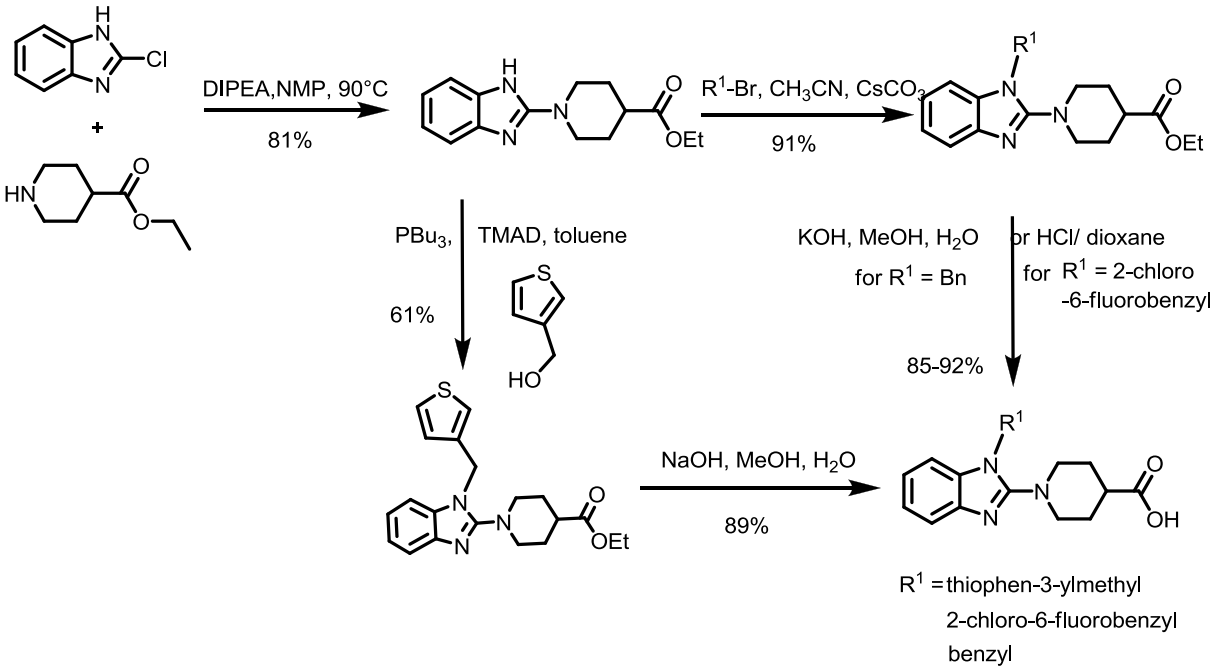
Supplementary scheme 6. Synthesis of benzimidazole fragments type B.



Supplementary Scheme 7. Synthesis of ether-linked benzimidazoles from fragments type A and B.

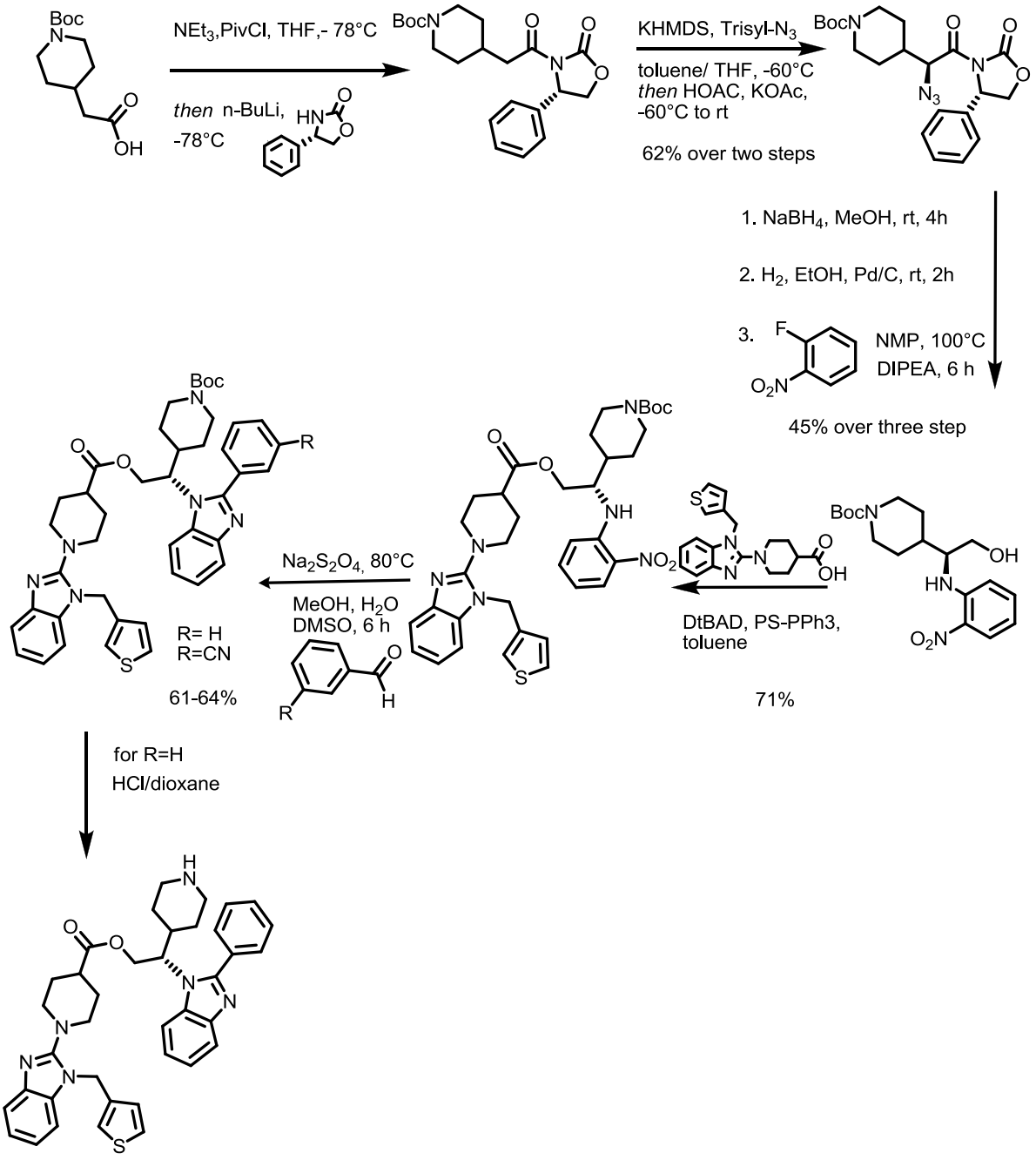
Synthesis of ester-linked benzimidazoles

Synthesis of benzimidazole-fragments type C.

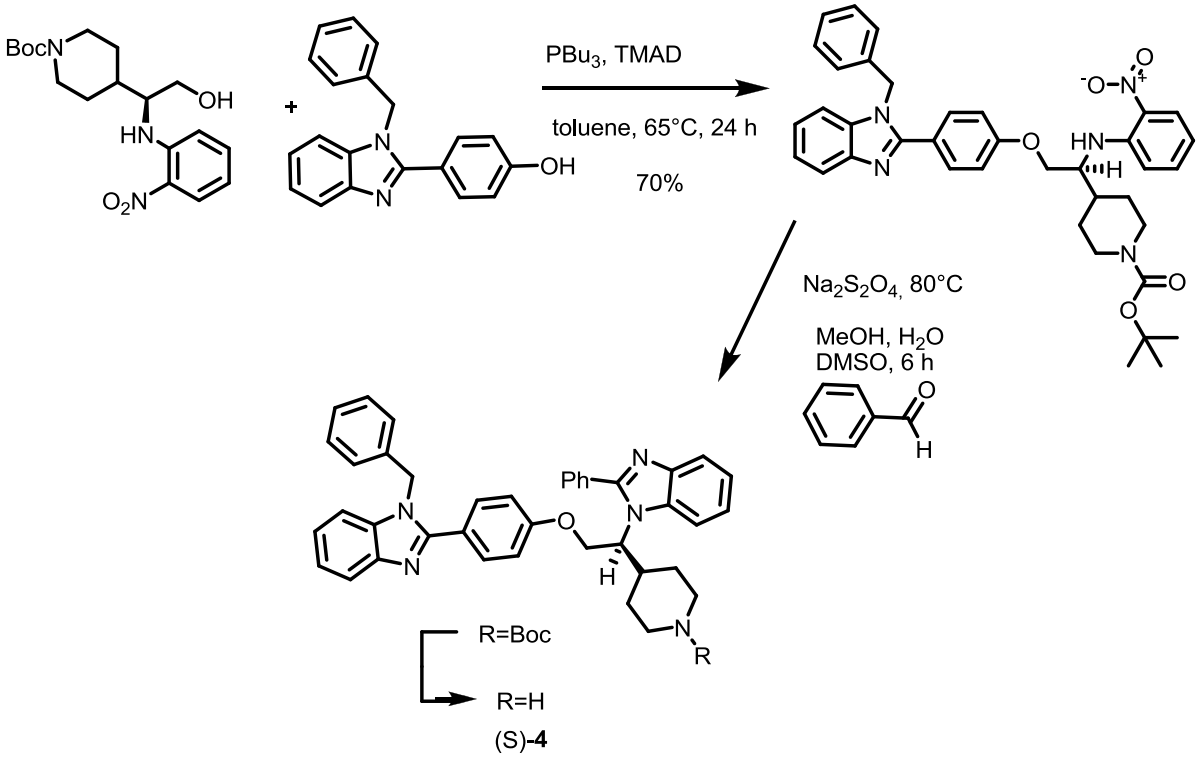


Supplementary scheme 8. Synthesis of benzimidazole-fragments type C.

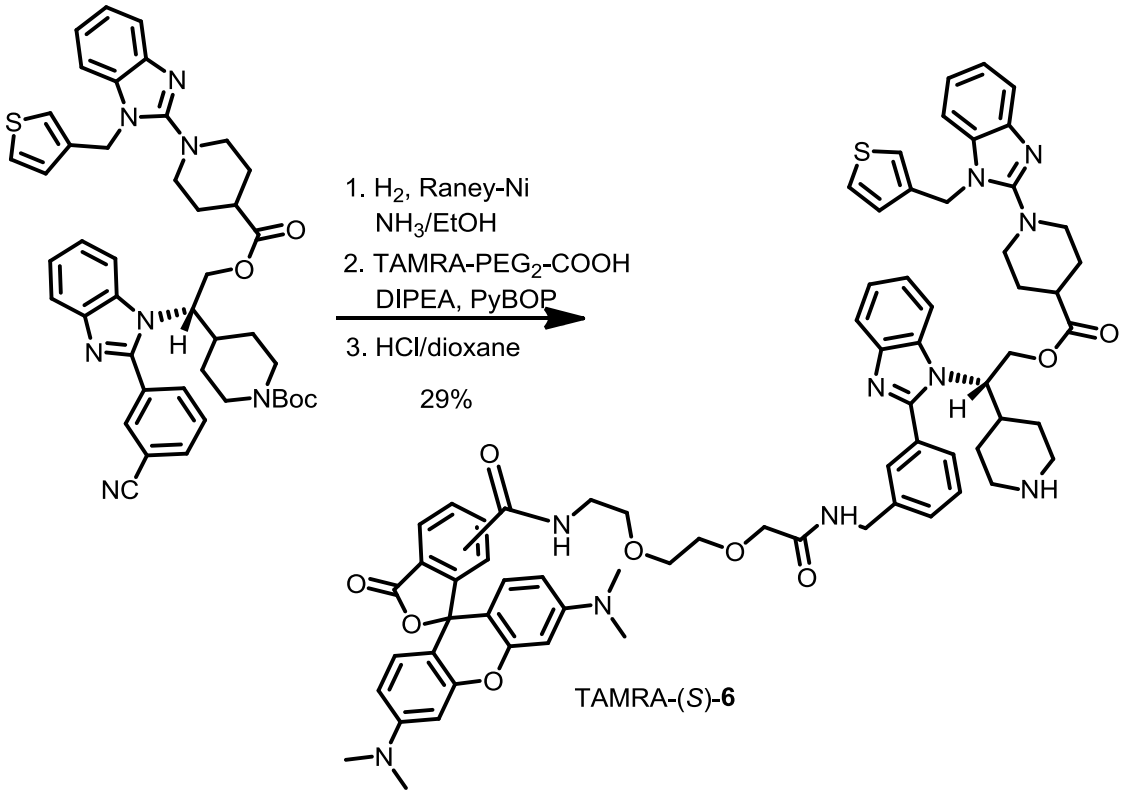
Enantioselective synthesis of Deltarin-(*S*)-4 and (*S*)-6



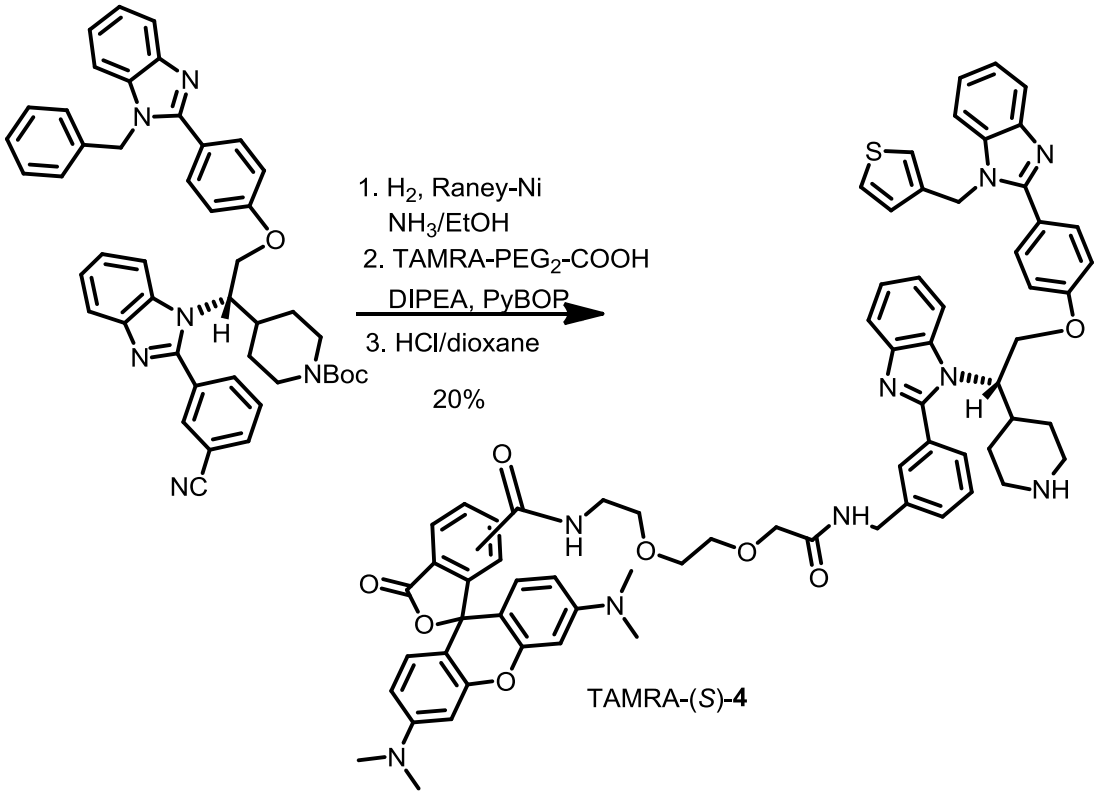
Supplementary scheme 9. Enantioselective synthesis of (*S*)-**6**



Supplementary scheme 10. Enantioselective synthesis of (*S*)-**4**

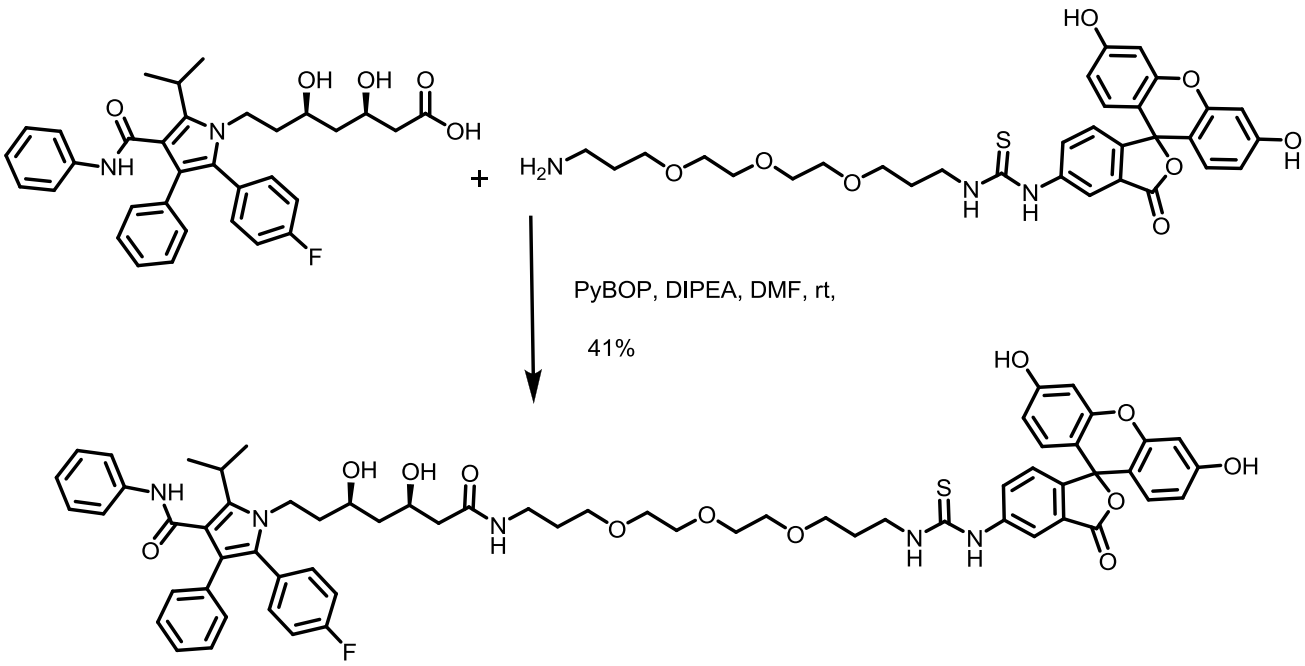


Supplementary scheme 11. Enantioselective synthesis of TAMRA-(*S*)-**6**



Supplementary scheme 12. Enantioselective synthesis of TAMRA-(*S*)-**4**

Synthesis of fluorescein-labeled Atorvastatin

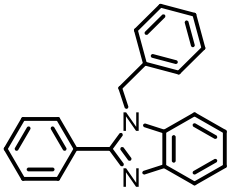


Supplementary scheme 13. Synthesis of fluorescein-labeled Atorvastatin

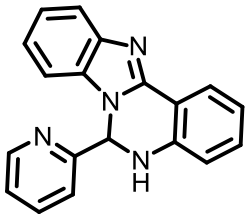
Experimental procedures for chemical synthesis

General Procedure (A) for the N-alkylation of benzimidazoles

To a suspension of benzimidazole in acetonitrile (1 mL/ 0.1 mM benzimidazole) was added caesium carbonate (1.5 eq) and the corresponding alkyl bromide (1.05 eq). The reaction mixture was stirred at room temperature for 1-3 hours and after this time it was concentrated *in vacuo*. The residue was suspended in a mixture of CH_2Cl_2 and sat. $NaHCO_3$ (vol % 50:50, 10 mL/0.1 mmol benzimidazole). The aqueous layer was extracted with CH_2Cl_2 (2 x, 10 mL/0.1 mmol benzimidazole). The combined organic layers were dried over Na_2SO_4, filtered and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography as described below.



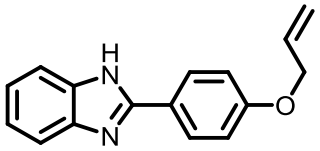
1-Benzyl-2-phenyl-1H-benzo[d]imidazole (1) The *N*-benzylation of 2-phenyl benzimidazole (185 mg, 0.92 mmol) was performed following the general procedure described above. The crude product was purified by flash column chromatography (gradient of cyclohexane : ethylacetate 10:1 to 1:1) to yield compound **1** (212 mg, 0.74 mmol, 86%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.76 – 7.67 (m, 3H), 7.57 – 7.47 (m, 3H), 7.47 – 7.42 (m, 1H), 7.31 – 7.16 (m, 5H), 6.98 (d, J = 7.3 Hz, 2H), 5.57 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 153.94, 143.37, 137.60, 136.57, 130.84, 130.50, 129.72, 129.45, 128.15, 126.77, 123.37, 122.89, 119.95, 111.78, 48.15. LC-MS (ESI): calcd for $C_{20}H_{16}N_2$: 285.13863 $[M+H]^+$, found 285.01 $[M+H]^+$, R_t = 5.90 min; HR-MS found 285.13883 $[M+H]^+$.



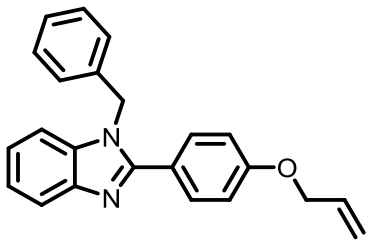
6-Pyiridyl-5,6-dihydrobenzo[4-5]imidazo[1,2-c]quinazoline (S1) A mixture of 2-pyridinecarboxaldehyde (99 mg, 0.93 mmol) and 2-(2-aminophenyl)-1*H*-benzimidazole (200 mg, 0.93 mmol) was dissolved in acetic acid (2 mL). The reaction mixture was stirred at 65°C for 16 h. After cooling to room temperature the reaction mixture was diluted

with CH_2Cl_2 (15 mL) and a sat. $NaHCO_3$ – solution was slowly added until a basic pH was reached. The aqueous layer was extracted with CH_2Cl_2 (2 x 15 mL). The combined organic phases were dried over Na_2SO_4, filtered and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (gradient of CH_2Cl_2: MeOH 50:1 to 5:1) to yield compound **2**. (196 mg, 0.66 mmol, 71%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.51 – 8.35 (m, 1H), 7.92 (d, *J* = 7.7 Hz, 1H), 7.73 (td, *J* = 6.1, 3.0 Hz, 1H), 7.69 (d, *J* = 2.0 Hz, 1H), 7.65 – 7.59 (m, 1H), 7.35 – 7.30 (m, 1H), 7.30 – 7.24 (m, 1H), 7.24 – 7.06 (m, 5H), 6.84 (d, *J* = 8.2 Hz, 1H), 6.81 – 6.70 (m, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 159.05, 150.14, 147.51, 144.46, 143.64, 138.10, 133.69, 132.20, 125.25, 124.61, 122.85, 122.70, 120.87, 119.25, 118.70, 115.36, 112.70, 111.07, 69.04. LC-MS (ESI): calcd for $C_{19}H_{14}N_4$: 299.12912 $[M+H]^+$, found 299.01 $[M+H]^+$, R_t = 2.70 min; HR-MS found 299.12929 $[M+H]^+$.

Procedures for synthesis of benzimidazole fragments A

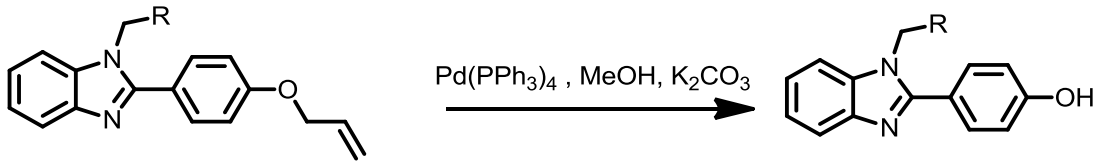


2-(4-(Allyloxy)phenyl)-1H-benzo[d]imidazole (S2) To a mixture of 4-allyloxybenzaldehyde (4.00 g, 23.9 mmol) and phenylendiamine (2.72 g, 25.1 mmol) in DMF (50 mL) at room temperature was added $Na_2S_2O_5$ (4.50 g, 23.9 mmol). The reaction mixture was heated at 70 °C for 3 h. To the reaction mixture was added ice water (150 mL) and the resulting suspension was stirred for 2 h at 0°C. The slightly brown precipitate was filtered, washed with cold water (100 mL), ethyl acetate (3x 20 mL) and dried *in vacuo* to afford the product. (4.85 g, 19.8 mmol, 81 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.11 (d, *J* = 8.7 Hz, 2H), 7.56 (dd, *J* = 5.9, 3.1 Hz, 2H), 7.23 – 7.06 (m, 4H), 6.11-5.99 (m, 1H), 5.41 (d, *J* = 16.8 Hz, 1H), 5.27 (d, *J* = 10.6 Hz, 1H), 4.64 (d, *J* = 4.7 Hz, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 160.3, 151.9, 139.7, 134.1, 128.8, 123.1, 122.6, 118.3, 115.8, 115.4, 69.02; LC-MS (ESI): calcd for $C_{16}H_{14}N_2O$: 251.11789 $[M+H]^+$, found 251.13 $[M+H]^+$, R_t = 5.84 min; HR-MS found 251.11800 $[M+H]^+$.

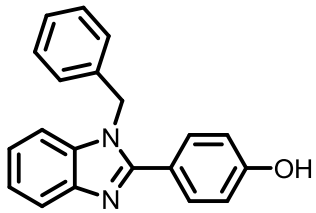


2-(4-(Allyloxy)phenyl)-1-benzyl-1H-benzo[d]imidazole (S3) Compound **S3** was synthesized according to Procedure (A) for the synthesis of N-alkylated benzimidazoles starting from **S2** (262 mg, 1.04 mmol). The crude product was purified by automated flash chromatography using a gradient of cyclohexane : ethyl acetate 10:1 to 1:2 to yield the benzimidazole (325 mg, 0.95 mmol, 91%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.70 – 7.60 (m, 3H), 7.43 – 7.37 (m, 1H), 7.30 – 7.14 (m, 5H), 7.07 (d, J = 8.8 Hz, 2H), 6.99 (d, J = 7.4 Hz, 2H), 6.11 – 5.93 (m, 1H), 5.55 (s, 2H), 5.40 (dd, J = 17.3, 1.7 Hz, 1H), 5.26 (dd, J = 10.5, 1.7 Hz, 1H), 4.62 (d, J = 5.2 Hz, 2H).^{13}C NMR (101 MHz, DMSO-d_6) δ 160.00, 153.89, 143.40, 137.68, 136.61, 134.08, 131.13, 129.47, 128.12, 126.72, 123.14, 123.07, 122.75, 119.72, 118.40, 115.60, 111.59, 68.99, 48.14. LC-MS (ESI): calcd for $C_{23}H_{20}N_2O$: 341.16484 $[M+H]^+$, found: 341.25 $[M+H]^+$, R_t = 7.43 min; HR-MS found 341.16508 $[M+H]^+$.

General Procedure (B) for the deprotection of allyl-protected phenols



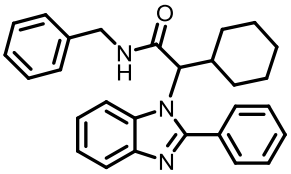
The allyl-protected phenol (1eq) was dissolved in degassed methanol (5 mL/mmol protected phenol). To this mixture was added K_2CO_3 (3 eq) and $Pd(PPh_3)_4$ (0.2 eq). The reaction mixture was stirred at room temperature for 1-3 hours and after this time it was concentrated *in vacuo*. The residue was resuspended in CH_2Cl_2 (10 mL/mmol) and a sat. NH_4Cl-solution was subsequently added. The pH of the aqueous layer was adjusted to 8 and the aqueous layer was extracted with CH_2Cl_2 (3 x 20 mL/mmol phenol). The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified as described below.



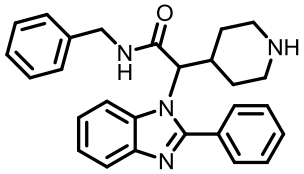
4-(1-Benzyl-1H-benzo[d]imidazol-2-yl)phenol (S4) Compound **S4** was synthesized according to Procedure B for the allyl-deprotection of benzimidazoles. The compound was purified by automated flash chromatography using a gradient of cyclohexane: ethyl acetate 20:1 to 1:1 to yield compound **S4** (143 mg, 0.47 mmol, 83%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.93 (s, 1H), 7.72 – 7.59 (m, 1H), 7.54 (d, J = 8.6 Hz, 2H), 7.41 – 7.33 (m, 1H), 7.32 –

7.12 (m, 5H), 6.99 (d, *J* = 7.4 Hz, 2H), 6.87 (d, *J* = 8.7 Hz, 2H), 5.53 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ = 160.3, 151.9, 139.7, 134.1, 128.8, 123.1, 122.6, 118.3, 115.7, 115.4, 69.0 LC-MS (ESI): calcd for $C_{20}H_{16}N_2O$: 301.13354 $[M+H]^+$, found: 301.18 $[M+H]^+$, R_t = 5.89 min; HR-MS found 301.13367 $[M+H]^+$.

Procedures for synthesis of benzimidazole fragments B

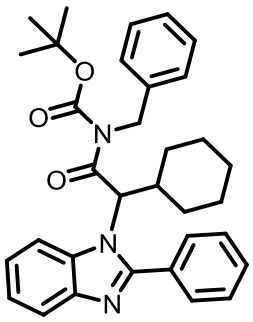


N-Benzyl-2-cyclohexyl-2-(2-phenyl-1H-benzo[d]imidazol-1-yl)acetamide (S5) A mixture of the aniline (1.80 g, 9.0 mmol) and cyclohexyl carbaldehyde (1.10 g, 9.9 mmol) was stirred at room temperature in MeOH (4 mL) for 10 minutes. To the reaction mixture was added benzylisocyanide (1.10 mL, 9 mmol) and a solution of benzoic acid (1.10 g 9.0 mmol) in MeOH (4 mL). The reaction mixture was stirred at room temperature for 36 h. After this time HCl in dioxane (4 M, 2 mL) was added to the reaction mixture. The reaction mixture was stirred for 36 h at room temperature and then the solvent was removed under reduced pressure. The residue was redissolved in CH_2Cl_2 (20 mL) and washed with a sat. $NaHCO_3$ solution (30 mL). The aqueous layer was extracted with CH_2Cl_2 (3x 20 mL). The combined organic layers were dried (Na_2SO_4), filtered and concentrated to dryness under reduced pressure. The residue was purified by flash chromatography using a gradient of cyclohexane :ethyl acetate 50:1 to 1:2 to afford the product (3.42 g, 8.06 mmol, 89 %,).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 – 7.86 (m, 1H), 7.81 – 7.69 (m, 1H), 7.56 – 7.35 (m, 5H), 7.36 – 7.19 (m, 5H), 7.10 (dd, *J* = 6.8, 2.4 Hz, 2H), 6.13 (s, 1H), 4.50 – 4.29 (m, 3H), 2.76 – 2.56 (m, 1H), 1.96 (d, *J* = 12.3 Hz, 1H), 1.71 – 1.41 (m, 3H), 1.36 – 1.18 (m, 1H), 1.12 – 0.86 (m, 3H), 0.86 – 0.69 (m, 1H), 0.66 – 0.46 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.50, 154.93, 143.37, 137.77, 134.33, 130.74, 130.24, 129.92, 129.14, 128.96, 127.91, 123.51, 123.12, 120.18, 113.97, 66.39, 43.97, 37.84, 31.36, 29.18, 26.13, 25.81. LC-MS (ESI): calcd for $C_{28}H_{29}N_3O$: 424.23834 $[M+H]^+$, found 424.21 $[M+H]^+$; HR-MS found 424.23733 $[M+H]^+$.



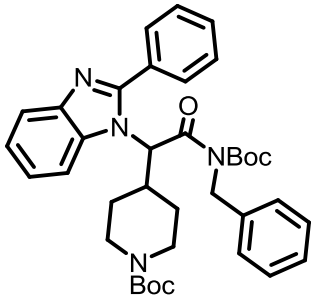
N-Benzyl-2-(2-phenyl-1H-benzo[d]imidazol-1-yl)-2-(piperidin-4-yl)acetamide (S6) A mixture of the aniline (1.20 g, 5.7 mmol) and the *tert*-butyl 4-formylpiperidine-1-carboxylate

(1.34 g, 6.3 mmol) was stirred at room temperature in MeOH (3 mL) for 10 minutes. To the reaction mixture was added benzylisocyanide (0.70 mL, 9.0 mmol) and a solution of benzoic acid (0.70 g 9.0 mmol) in MeOH (4 mL). The reaction mixture was stirred at room temperature for 36 h. To the reaction mixture was added a HCl/dioxane (4 M, 2 mL). The reaction mixture was stirred for 36 h at room temperature and then concentrated *in vacuo*. The residue was redissolved in CH_2Cl_2 (20 mL) and washed with sat. $NaHCO_3$ solution (30 mL). The aqueous layer was reextracted with CH_2Cl_2 (3x 20 mL). The combined organic layers were dried (Na_2SO_4), filtered and concentrated to dryness under reduced pressure. The residue was purified by automated flash chromatography (gradient cyclohexane : ethylacetate 100:1 to 1:2, containing 0.1% triethylamine) to afford the product (4.11 mmol,1.75 g, 72%).
^{1}H NMR (400 MHz, DMSO-d_6) δ 8.89 (t, J = 5.6 Hz, 1H), 8.10 – 7.89 (m, 1H), 7.68 – 7.47 (m, 6H), 7.34 – 7.17 (m, 7H), 2.84 (d, J = 11.5 Hz, 1H), 2.67 – 2.51 (m, 2H), 2.37 (t, J = 12.4 Hz, 1H), 2.08 (t, J = 11.2 Hz, 1H), 1.60 (d, J = 13.0 Hz, 1H), 1.12 – 0.97 (m, 1H), 0.62 – 0.48 (m, 1H), 0.47 – 0.36 (m, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.11, 155.19, 154.32, 152.61, 143.70, 138.15, 131.13, 130.49, 130.22, 129.37, 129.03, 127.91, 127.51, 123.29, 122.95, 120.05, 115.57, 84.79, 79.34, 63.36, 48.55, 37.71, 28.72, 27.85. LC-MS (ESI): calcd for $C_{27}H_{28}N_4O$: 425.23376 $[M+H]^+$, found 425.17 $[M+H]^+$, R_t = 5.15 min; HR-MS found 425.23359 $[M+H]^+$.

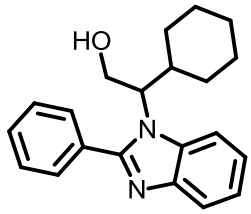


tert-Butyl benzyl(2-cyclohexyl-2-(2-phenyl-1H-benzo[d]imidazol-1-yl)acetyl)carbamate (S7) To a solution of amide **S5** (620 mg, 1.46 mmol) in CH_2Cl_2 (5 mL) was added Boc-anhydride (639 mg, 2.92 mmol), triethylamine (405 µL, 2.92 mmol) and DMAP (179 mg, 1.46 mmol). The reaction mixture was stirred at room temperature for 5 h. After this time Boc-anhydride (639 mg, 2.92 mmol), triethylamine (405 µL, 2.92 mmol) and DMAP (179 mg, 1.46 mmol) were added to the reaction mixture and stirring was continued for an additional 12 h. The reaction mixture was concentrated to dryness under reduced pressure. Purification by automated flash chromatography using a gradient of cyclohexane : ethyl acetate 20:1 to 1:1 afforded the product (0.66 g, 1.26 mmol, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, J = 7.4 Hz, 1H), 7.82 – 7.73 (m, 1H), 7.56 – 7.48 (m, 2H), 7.47 – 7.39 (m,

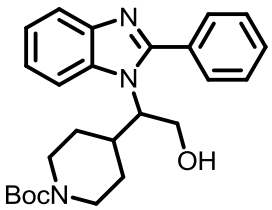
3H), 7.34 – 7.24 (m, 2H), 7.24 – 7.15 (m, 3H), 7.05 (d, *J* = 7.2 Hz, 2H), 6.38 (d, *J* = 10.6 Hz, 1H), 4.83 (d, *J* = 15.0 Hz, 1H), 4.68 (d, *J* = 14.9 Hz, 1H), 2.87 (d, *J* = 10.8 Hz, 1H), 1.84 – 1.68 (m, 2H), 1.72 – 1.50 (m, 2H), 1.38 – 0.74 (m, 15H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.81, 155.26, 152.49, 143.59, 137.78, 134.92, 131.22, 130.10, 129.81, 128.75, 128.56, 127.55, 127.42, 123.03, 122.68, 120.04, 115.08, 84.19, 64.13, 48.53, 39.52, 29.87, 29.66, 27.95, 26.38, 26.25, 26.16. LC-MS (ESI): calcd for $C_{33}H_{37}N_3O_3$: 524.29077 $[M+H]^+$, found 524.18 $[M+H]^+$, R_t = 11.55 min; HR-MS found 524.29035 $[M+H]^+$.



tert-Butyl 4-(2-(benzyl(tert-butoxycarbonyl)amino)-2-oxo-1-(2-phenyl-1H-benzo[d]imidazol-1-yl)ethyl)piperidine-1-carboxylate (S8) To a solution of amide **S7** (300 mg, 0.71 mmol) in CH_2Cl_2 (5 mL) was added Boc-anhydride (463 mg, 2.13 mmol), triethylamine (293 µL, 2.13 mmol) and DMAP (86 mg, 0.71 mmol). The reaction mixture was stirred at room temperature for 5 h. Boc-anhydride (463 mg, 2.13 mmol), triethylamine (293 µL, 2.13 mmol) and DMAP (86 mg, 0.71 mmol) were added to the reaction mixture and stirring was continued for an additional 12 h. The reaction mixture was concentrated to dryness under reduced pressure. Purification by flash chromatography using a gradient of cyclohexane: ethyl acetate 20:1 to 1:1 afforded the product (0.36 g, 0.58 mmol, 81%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.04 (d, *J* = 7.5 Hz, 1H), 7.65 (d, *J* = 8.0 Hz, 1H), 7.55 – 7.37 (m, 5H), 7.31 – 7.10 (m, 5H), 7.04 (d, *J* = 7.2 Hz, 2H), 6.23 (d, *J* = 10.4 Hz, 1H), 4.77 (d, *J* = 15.5 Hz, 1H), 4.64 (d, *J* = 15.1 Hz, 1H), 3.95 (d, *J* = 11.9 Hz, 1H), 3.82 (d, *J* = 11.2 Hz, 1H), 2.97 (d, *J* = 10.2 Hz, 1H), 2.86 – 2.59 (m, 1H), 2.56 – 2.40 (m, 2H), 1.59 (d, *J* = 11.7 Hz, 1H), 1.47 – 1.24 (m, 10H), 1.25 – 1.05 (m, 9H), 1.05 – 0.88 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.11, 155.19, 154.32, 152.61, 143.70, 138.15, 131.13, 130.49, 130.22, 129.37, 129.03, 127.91, 127.51, 123.29, 122.95, 120.05, 115.57, 84.79, 79.34, 63.36, 48.55, 37.71, 28.72, 27.85. LC-MS (ESI): calcd for $C_{37}H_{44}N_4O_5$: 625.33845 $[M+H]^+$, found 625.19 $[M+H]^+$, R_t = 10.76 min; HR-MS found 625.33840.

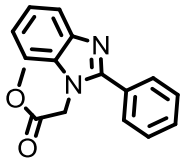


2-Cyclohexyl-2-(2-phenyl-1H-benzo[d]imidazol-1-yl)ethanol (S9) A solution of Boc-protected amide **S7** (650 mg, 1.24 mmol) in EtOH:THF (1:5, 5 mL) was added to a suspension of $NaBH_4$ (188 mg, 5 mmol) in THF (4 mL) at room temperature over 1 h. The reaction mixture was stirred at room temperature for 12 h. After this time $NaBH_4$ (94 mg, 2.5 mmol) was added to the reaction mixture and stirring was continued for 3 h. To the reaction mixture was added a NH_4Cl-solution (15 mL), followed by CH_2Cl_2 (20 mL). The pH of the aqueous layer was adjusted to 9 by addition of sat. $NaHCO_3$-solution. The aqueous layers were extracted with CH_2Cl_2 (2x 20 mL). The combined organic layers were dried (Na_2SO_4), filtered and concentrated under reduced pressure and the residue was purified by flash chromatography (gradient cyclohexane: ethyl acetate 10:1 to 1:1) to afford the product (330 mg, 1.03 mmol, 83%).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 – 7.61 (m, 3H), 7.52 (d, *J* = 8.1 Hz, 1H), 7.48 – 7.35 (m, 3H), 7.25 – 7.10 (m, 2H), 4.36 – 4.21 (m, 2H), 4.06 – 4.02 (m, 1H), 3.13 (s, 1H), 2.18 – 2.05 (m, 1H), 1.80 (d, *J* = 11.9 Hz, 1H), 1.67 (d, *J* = 13.6 Hz, 1H), 1.53 (d, *J* = 10.0 Hz, 1H), 1.41 (d, *J* = 12.5 Hz, 1H), 1.31 – 1.10 (m, 1H), 1.04 – 0.77 (m, 4H), 0.57 – 0.37 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.21, 143.34, 133.52, 130.86, 130.56, 129.69, 128.63, 122.62, 122.51, 120.24, 112.34, 65.04, 61.68, 37.67, 30.88, 29.62, 26.06, 25.87, 25.74. LC-MS (ESI): calcd for $C_{37}H_{44}N_4O_5$: 321.19614 $[M+H]^+$, found 321.18 $[M+H]^+$, R_t = 6.24 min; HR-MS found 321.19642.

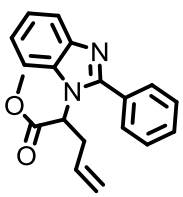


tert-Butyl 4-(2-hydroxy-1-(2-phenyl-1H-benzo[d]imidazol-1-yl)ethyl)piperidine-1-carboxylate (S10) A solution of Boc-protected amide **S8** (1.0 g, 1.6 mmol) in EtOH:THF (1:5, 15 mL) was added to a suspension of $NaBH_4$ (242 mg, 6.4 mmol) in THF (15 mL) at room temperature over 1 h. The reaction mixture was stirred at room temperature for 12 h. $NaBH_4$ (120 mg, 3.2 mmol) was added to the reaction mixture and stirring was continued for

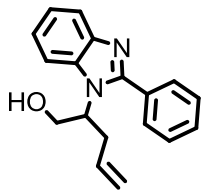
3 h. To the reaction mixture was added NH_4Cl-solution (40 mL), followed by CH_2Cl_2 (30 mL). The pH of the aqueous layer was adjusted to 8 by addition of sat. $NaHCO_3$-solution. The aqueous layers were reextracted with CH_2Cl_2 (3x 30 mL). The combined organic layers were concentrated *in vacuo* and the residue was purified by flash chromatography (gradient cyclohexane: ethyl acetate 10:1 to 1:2) to afford the product (538 mg, 1.27 mmol, 80%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.87 – 7.78 (m, 1H), 7.74 (d, J = 3.6 Hz, 2H), 7.70 – 7.62 (m, 1H), 7.58 – 7.46 (m, 3H), 7.26 – 7.11 (m, 2H), 5.29 – 5.07 (m, 1H), 4.30 – 4.07 (m, 2H), 4.04 – 3.73 (m, 2H), 3.71 – 3.44 (m, 1H), 2.79 – 2.56 (m, 1H), 2.45 – 2.23 (m, 2H), 1.81 (d, J = 12.2 Hz, 1H), 1.46 – 1.20 (m, 10H), 0.93 (d, J = 10.4 Hz, 1H), 0.68 – 0.38 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 155.95, 154.28, 143.86, 134.19, 131.67, 130.82, 130.09, 129.08, 122.74, 122.40, 120.11, 113.62, 79.18, 64.71, 60.33, 35.71, 30.18, 29.06, 28.67.LC-MS (ESI): calcd for $C_{25}H_{31}N_3O_3$: 422.24382 $[M+H]^+$, found 422.20 $[M+H]^+$, R_t = 6.31 min; HR-MS found 422.24271.



Methyl 2-(2-phenyl-1H-benzo[d]imidazol-1-yl)acetate (S11) To a suspension of 2-phenyl benzimidazole (0.32 g, 1.6 mmol) in acetonitrile (5 mL) was added caesium carbonate (0.80 g, 2.5 mmol) and methyl bromoacetate (0.16 mL, 1.7 mmol). The reaction mixture was stirred at room temperature for 3 hours and concentrated *in vacuo*. The residue was suspended in a mixture of CH_2Cl_2 and sat. $NaHCO_3$ (vol % 50:50, 15 mL). The aqueous layer was reextracted with CH_2Cl_2 (2 x 20 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated in *in vacuo* to afford the product (0.40 g, 1.5 mmol, 91%). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 7.77 – 7.63 (m, 3H), 7.63 – 7.44 (m, 4H), 7.33 – 7.09 (m, 2H), 5.21 (s, 2H), 3.64 (s, 3H). ^{13}C NMR (101 MHz, DMSO-*d6*) δ 169.40, 153.91, 143.08, 136.91, 130.57, 130.51, 129.62, 129.53, 123.45, 123.00, 119.87, 111.36, 53.17, 46.57. LC-MS (ESI): calcd for $C_{16}H_{14}N_2O_2$: 267.11280 $[M+H]^+$, found: 267.18 [M+H]+; HR-MS found 267.11311 $[M+H]^+$.

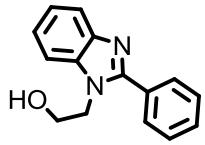


Methyl 2-(2-phenyl-1H-benzo[d]imidazol-1-yl)pent-4-enoate (S12) To a solution of **S11** (500 mg, 1.88 mmol) in DMF (5 mL) was added at 0°C NaH (83 mg, 60% mineral oil suspension). The reaction mixture was stirred for 60 minutes, then a solution of allyl bromide (244 µL, 2.90 mmol) in DMF (2 mL) was added. The reaction mixture was allowed to warm to room temperature and stirred for 3 h. A saturated solution of NH_4Cl (10 mL) was added at 0°C to the reaction mixture. The aqueous suspension was extracted with CH_2Cl_2 (3 x 20 mL). The combined organic phases were dried over Na_2SO_4, filtered and concentrated to dryness. The crude product was purified by automated flash chromatography using a of gradient cyclohexane : ethyl acetate 10:1 to 1:3 to yield the product (340 mg, 1.11 mmol, 59%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.71 – 7.66 (m, 1H), 7.65 – 7.59 (m, 2H), 7.60 – 7.53 (m, 3H), 7.51 – 7.43 (m, 1H), 7.30 – 7.19 (m, 2H), 5.36 (dd, J = 9.5, 6.3 Hz, 1H), 5.32 – 5.16 (m, 1H), 4.76 (d, J = 10.2 Hz, 1H), 4.65 (d, J = 17.1 Hz, 1H), 3.68 (s, 3H), 2.99 – 2.77 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.08, 154.65, 143.50, 134.57, 133.12, 130.73, 130.61, 130.17, 129.53, 123.50, 122.94, 120.30, 119.37, 112.40, 58.12, 53.61, 33.85. LC-MS (ESI): calcd for $C_{19}H_{18}N_2O_2$: 307.14410 $[M+H]^+$, found: 307.16 $[M+H]^+$; HR-MS found 307.14440 $[M+H]^+$.



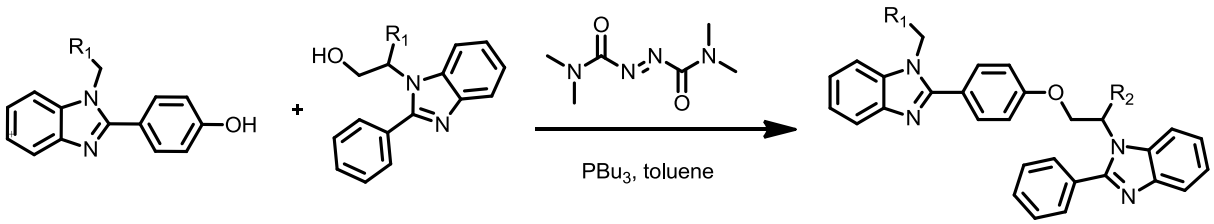
2-(2-Phenyl-1H-benzo[d]imidazol-1-yl)pent-4-en-1-ol (S13) A solution of benzimidazole **S12** (320 mg, 0.94 mmol) in EtOH:THF (1:5, 5 mL) was added to a suspension of $NaBH_4$ (188 mg, 5 mmol) in THF (4 mL) at room temperature over 1 h. The reaction mixture was stirred at room temperature for 12 h. $NaBH_4$ (94 mg, 2.5 mmol) was added to the reaction mixture and stirring was continued for 3 h. To the reaction mixture was added sat. NH_4Cl-solution (15 mL), followed by CH_2Cl_2 (30 mL). The pH of the aqueous layer was adjusted to 9 by addition of sat. $NaHCO_3$-solution. The aqueous layer was again extracted with CH_2Cl_2 (2x 20 mL). The combined organic layers were dried (Na_2SO_4), filtered, concentrated to dryness and the residue was purified by flash chromatography (gradient cyclohexane : ethyl acetate 100:1 to 1:1) to afford the product (217 mg, 0.77 mmol, 83%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 – 7.62 (m, 1H), 7.63 – 7.56 (m, 2H), 7.47 (d, J = 7.9 Hz, 1H), 7.44 – 7.30 (m, 3H), 7.27 – 7.11 (m, 2H), 5.41 – 5.19 (m, 1H), 4.88 – 4.65 (m, 2H), 4.65 – 4.45 (m, 1H), 4.35 – 4.10 (m, 1H), 3.94 – 3.76 (m, 1H), 2.85 – 2.62 (m, 1H), 2.57 – 2.43 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.69, 143.40, 133.48, 133.05, 130.70, 130.37, 129.78, 128.62, 122.64,

122.53, 120.20, 118.56, 112.31, 62.97, 59.55, 34.09. LC-MS (ESI): calcd for $C_{18}H_{18}N_2O$: 279.14919 $[M+H]^+$, found: 279.10 $[M+H]^+$; HR-MS found 279.14941 $[M+H]^+$.



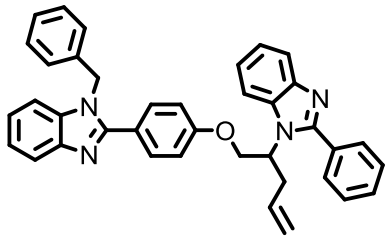
2-(2-Phenyl-1H-benzo[d]imidazol-1-yl)ethanol (S14) To a solution of benzimidazole **S11** (400 mg, 1.43 mmol) in MeOH (5 mL) was added $NaBH_4$ (376 mg, 10.0 mmol) at room temperature. The reaction mixture was stirred at room temperature for 12 h. To the reaction mixture was added sat. NH_4Cl-solution (15 mL), followed by CH_2Cl_2 (30 mL). The pH of the aqueous layer was adjusted to 8 by addition of sat. $NaHCO_3$-solution. The aqueous layer was reextracted with CH_2Cl_2 (2x 20 mL). The combined organic layers were dried (Na_2SO_4), filtered and concentrated *in vacuo* and the residue was purified by flash chromatography (gradient cyclohexane : ethyl acetate 100:1 to 1:1) to afford the product (302 mg, 1.26 mmol, 84%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.97 – 7.84 (m, 2H), 7.76 – 7.60 (m, 2H), 7.60 – 7.45 (m, 3H), 7.34 – 6.99 (m, 2H), 5.08 (s, 1H), 4.33 (t, J = 5.5 Hz, 2H), 3.89 – 3.56 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 154.25, 143.32, 136.56, 131.27, 130.32, 130.20, 129.21, 122.93, 122.56, 119.71, 111.89, 59.92, 47.49. LC-MS (ESI): calcd for $C_{15}H_{14}N_2O$: 239.11789 $[M+H]^+$, found: 239.19 $[M+H]^+$, Rt = 4.32 min; HR-MS found 239.11806 $[M+H]^+$.

General Procedure C for the synthesis of phenol ethers via Mitsunobu reaction

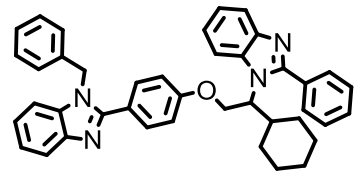


To a mixture of phenol, alcohol (1.0 eq) and tri-*n*-butyl phosphine (2 eq) in toluene (3 mL/mmol phenol) was added at 0°C TMAD (2 eq) in one portion. The resulting suspension was heated for 16 h at 60°C. If the reaction was not complete tri-*n*-butyl phosphine (1 eq) and TMAD (1 eq) were added at room temperature and the mixture was heated for 16 h at 60°C. The reaction mixture was diluted with CH_2Cl_2 (20 mL/mmol phenol) and washed with sat. $NaHCO_3$ (10 mL /mmol phenol). The aqueous phase was reextracted with CH_2Cl_2 (3 x 20 mL/mmol phenol). The combined organic phases were dried (Na_2SO_4),

filtered and concentrated *in vacuo*. The crude product was purified by column chromatography as described below.

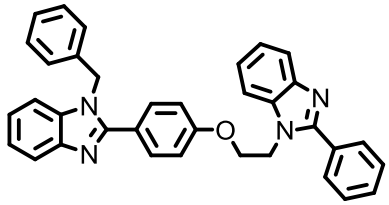


1-Benzyl-2-(4-(2-(2-phenyl-1H-benzo[d]imidazol-1-yl)pent-4-enyloxy)phenyl)-1H-benzo[d]imidazole (2) The product was synthesized from phenol **S4** (76 mg, 0.25 mmol) and alcohol **S13** according to Procedure C for the synthesis of phenol ethers via Mitsunobu reaction. The crude product was purified by automated flash chromatography using a gradient of of cyclohexane: ethyl acetate 10:1 to 1:7 to yield the product (100 mg, 0.18 mmol, 72 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.99 – 7.89 (m, 1H), 7.75 – 7.63 (m, 4H), 7.57 (t, *J* = 11.0 Hz, 2H), 7.54 – 7.43 (m, 3H), 7.38 (dt, *J* = 8.8, 4.5 Hz, 1H), 7.34 – 7.06 (m, 8H), 6.98 (dd, *J* = 7.7, 5.4 Hz, 4H), 5.51 (s, 2H), 5.50 – 5.33 (m, 1H), 4.91 – 4.71 (m, 4H), 4.44 (dd, *J* = 10.1, 3.6 Hz, 1H), 3.08 – 2.91 (m, 1H), 2.81 – 2.68 (m, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 159.51, 155.59, 153.74, 143.91, 143.37, 137.66, 136.60, 133.94, 133.85, 131.47, 131.14, 130.59, 130.20, 129.47, 129.18, 128.11, 126.66, 123.48, 123.12, 123.01, 122.78, 122.61, 120.29, 119.73, 119.09, 115.33, 113.31, 111.59, 68.29, 56.84, 48.12, 33.72. LC-MS (ESI): calcd for $C_{38}H_{32}N_4O$: 561.26489 $[M+H]^+$, found: 561.36 $[M+H]^+$, Rt = 7.19 min; HR-MS found 561.26523 $[M+H]^+$.

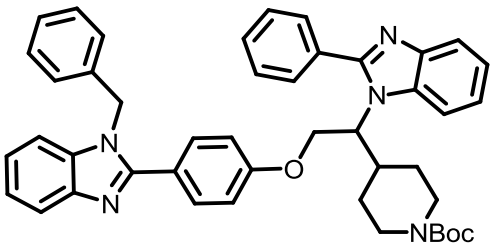


1-Benzyl-2-(4-(2-cyclohexyl-2-(2-phenyl-1H-benzo[d]imidazol-1-yl)ethoxy)phenyl)-1H-benzo[d]imidazole (3) The product was synthesized from phenol **S4** (50 mg, 0.17 mmol) and alcohol **S9** according to Procedure C for the synthesis of phenol ethers via Mitsunobu reaction. The crude product was purified by automated flash chromatography using a gradient of of cyclohexane: ethyl acetate 10:1 to 1:7 to yield the product (63 mg, 0.11 mmol, 63%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.91 (d, *J* = 7.3 Hz, 1H), 7.73 (d, *J* = 6.9 Hz, 2H), 7.66 (td, *J* = 7.1, 4.6 Hz, 2H), 7.58 (t, *J* = 10.6 Hz, 2H), 7.57 – 7.49 (m, 3H), 7.39 (d, *J* = 7.6 Hz, 1H), 7.28 – 7.15 (m, 7H), 7.04 (d, *J* = 8.6 Hz, 2H), 6.96 (d, *J* = 7.4 Hz, 2H), 5.51 (s, 2H), 4.85 (t, *J* = 9.9 Hz, 1H), 4.61 – 4.54 (m, 1H), 4.45 (t, *J* = 8.4 Hz, 1H), 2.37 (d, *J* = 10.8 Hz, 1H), 1.99 – 1.92

(m, 1H), 1.64 (d, *J* = 13.2 Hz, 1H), 1.53 – 1.35 (m, 3H), 1.28 – 1.17 (m, 1H), 1.05 – 0.75 (m, 4H), 0.59 – 0.49 (m, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 159.71, 155.88, 153.73, 143.76, 143.29, 137.68, 136.58, 133.93, 131.13, 130.66, 130.27, 129.47, 129.28, 128.10, 126.64, 123.39, 123.13, 123.06, 122.80, 122.63, 120.24, 119.68, 115.39, 113.35, 111.61, 109.99, 67.38, 62.19, 48.08, 37.36, 30.74, 29.64, 26.11, 25.84, 25.79. LC-MS (ESI): calcd for $C_{41}H_{38}N_4O$: 603.31184 $[M+H]^+$, found: 603.39 $[M+H]^+$, R_t = 8.14 min; HR-MS found 603.31156 $[M+H]^+$.



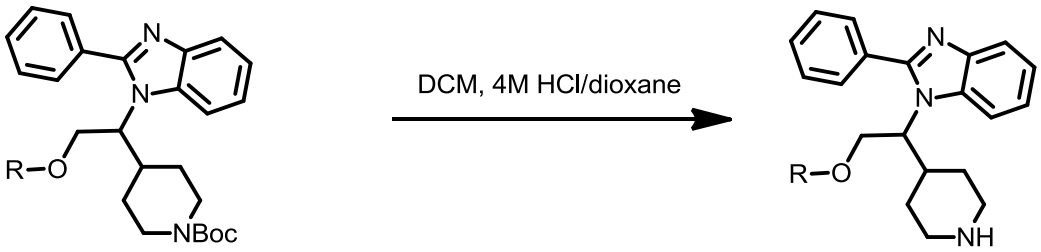
1-Benzyl-2-(4-(2-(2-phenyl-1H-benzo[d]imidazol-1-yl)ethoxy)phenyl)-1H-benzo[d]imidazole (S15) The product was synthesized from phenol **S4** (50 mg, 0.17 mmol) and alcohol **S14** according to Procedure C for the synthesis of phenol ethers via Mitsunobu reaction. The crude product was purified by automated flash chromatography using a gradient of of cyclohexane: ethyl acetate 6:1 to 1:9 to yield the product (52 mg, 0.10 mmol, 59%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.86 – 7.79 (m, 2H), 7.77 (d, *J* = 7.9 Hz, 1H), 7.70 – 7.62 (m, 2H), 7.60 – 7.45 (m, 5H), 7.38 (d, *J* = 6.8 Hz, 1H), 7.33 – 7.13 (m, 7H), 6.96 (d, *J* = 7.2 Hz, 2H), 6.86 (d, *J* = 8.7 Hz, 2H), 5.49 (s, 2H), 4.70 (t, *J* = 5.0 Hz, 2H), 4.36 (t, *J* = 5.1 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 159.51, 154.48, 153.74, 143.35, 137.64, 136.56, 136.25, 131.22, 131.09, 130.27, 129.45, 129.29, 128.10, 126.68, 123.36, 123.14, 123.10, 122.77, 119.84, 119.71, 115.17, 111.94, 111.59, 66.71, 48.09, 44.41. LC-MS (ESI): calcd for $C_{35}H_{28}N_4O$: 521.23359 $[M+H]^+$, found: 521.34 $[M+H]^+$, Rt = 6.37 min; HR-MS found 521.23314 $[M+H]^+$.



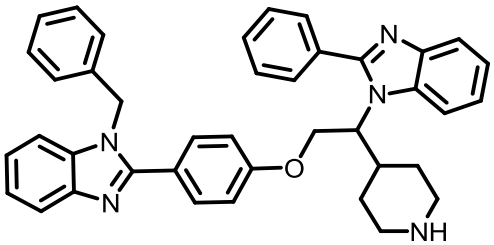
tert-Butyl-4-(2-(4-(1-benzyl-1H-benzo[d]imidazol-2-yl)phenoxy)-1-(2-phenyl-1H-benzo[d]imidazol-1-yl)ethyl)piperidine-1-carboxylate (S16) The product was synthesized from phenol **S4** (60 mg, 0.14 mmol) and alcohol **S10** according to Procedure C for the synthesis of phenol ethers via Mitsunobu reaction. The crude product was purified by

automated flash chromatography using a gradient of of cyclohexane: ethyl acetate 10:1 to 1:7 to yield the product (66 mg, 0.09 mmol, 66%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.94 (d, J = 6.3 Hz, 1H), 7.78 – 7.64 (m, 4H), 7.64 – 7.49 (m, 5H), 7.40 (d, J = 7.9 Hz, 1H), 7.31 – 7.15 (m, 7H), 7.04 (d, J = 8.2 Hz, 2H), 6.97 (d, J = 7.2 Hz, 2H), 5.51 (s, 2H), 4.85 (d, J = 9.5 Hz, 1H), 4.58 (d, J = 9.6 Hz, 1H), 4.48 (s, 1H), 3.91 (d, J = 12.6 Hz, 1H), 3.70 (s, 1H), 2.80 – 2.52 (m, 2H), 2.45 – 2.31 (m, 1H), 1.92 (d, J = 11.6 Hz, 1H), 1.31 (s, 9H), 1.19-1.00 (d, J = 9.6 Hz, 2H), 0.92 – 0.63 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 159.68, 155.83, 154.29, 153.69, 143.76, 143.18, 137.64, 136.56, 131.16, 130.69, 130.31, 129.46, 129.29, 128.11, 126.66, 123.39, 123.17, 122.84, 122.75, 120.29, 119.65, 115.42, 113.29, 111.62, 79.30, 67.12, 61.64, 48.13, 35.89, 29.79, 29.08, 28.71. LC-MS (ESI): calcd for $C_{45}H_{45}N_5O_3$: 704.35952 $[M+H]^+$, found: 704.31 $[M+H]^+$, R_t = 7.68 min; HR-MS found 704.36024 $[M+H]^+$. Chiral, analytical HPLC conditions: flow rate: 0.5 mL/min; solvent: 40 % ethanol in *iso*-hexane. (*S*)- **S16** R_t =90.1 min, (*R*)- **S16** 100.9 min.

General Procedure (D) for the Boc-deprotection of piperidine-containing benzimidazoles



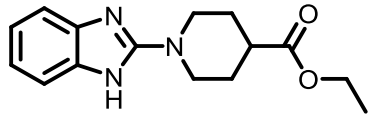
To a solution of Boc-protected piperidine in DCM (1 mL / 20 µM protected piperidine) was added a 4 M HCl/dioxane solution to a final concentration of 2 M HCl. The reaction mixture was stirred at room temperature for 3 h. The solvent was removed *in vacuo* to afford the product in quantitative yield as an HCl-salt. Further purification, if required, is described below.



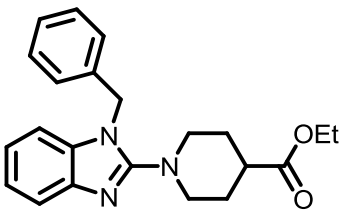
1-Benzyl-2-(4-(2-(2-phenyl-1H-benzo[d]imidazol-1-yl)-2-(piperidin-4-yl)ethoxy)phenyl)-1H-benzo[d]imidazole (4) The product was synthesized according to General Procedure (D) starting from (32 mg, 0.046 mmol) to afford the HCl-salt in quantitative yield. The residue

was dissolved in methanol (500 µL) and applied to a preparative C18-RP column eluting with a gradient (CH_3CN: H_2O 1:5 to 9:1 containing 0.1% TFA), to afford the desired piperdine (20 mg, 0.033 mmol, 71%) as a TFA-salt. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.92 (d, J = 6.8 Hz, 1H), 7.77 – 7.71 (m, 2H), 7.69 – 7.63 (m, 2H), 7.63 – 7.49 (m, 5H), 7.41 – 7.36 (m, 1H), 7.30 – 7.14 (m, 7H), 7.05 (d, J = 8.6 Hz, 2H), 6.97 (d, J = 7.3 Hz, 2H), 5.51 (s, 2H), 4.86 (t, J = 9.9 Hz, 1H), 4.62 – 4.52 (m, 1H), 4.47 (t, J = 8.5 Hz, 1H), 2.89 (d, J = 12.0 Hz, 1H), 2.68 (d, J = 12.0 Hz, 1H), 2.46 – 2.34 (m, 1H), 2.28 – 2.11 (m, 1H), 1.89 – 1.80 (m, 1H), 1.13 – 0.98 (m, 1H), 0.77 – 0.51 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 159.70, 155.88, 153.74, 143.78, 143.36, 137.68, 136.61, 133.94, 131.49, 131.14, 130.68, 130.27, 129.46, 129.28, 128.10, 126.65, 123.48, 123.10, 122.78, 122.66, 120.27, 119.71, 115.40, 113.27, 111.58, 67.15, 62.29, 48.11, 46.11, 46.01, 36.24, 31.25, 29.88. LC-MS (ESI): calcd for $C_{40}H_{37}N_5O$: 604.30709 $[M+H]^+$, found: 604.26 $[M+H]^+$, R_t = 5.34 min; HR-MS found: 604.30716 $[M+H]^+$.

Procedures for the synthesis of benzimidazole fragments C

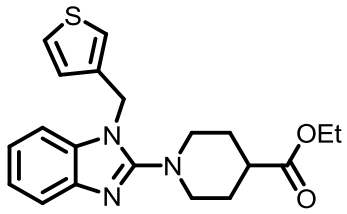


Ethyl 1-(1H-benzo[d]imidazol-2-yl)piperidine-4-carboxylate (S17) To a solution of 2-chloro benzimidazole (6.0 g, 39.3 mmol) in NMP (40 mL) was added 4-carboxyethyl piperidine (16.5 g, 105 mmol) and diisopropylethylamine (13.4 mL, 78.2 mmol). The reaction mixture was heated to 90°C and stirred for 16 h. Water (100 mL) was added and stirring was continued for an additional 20 min. The precipitate was collected by filtration and washed with water (3x 100 mL) and EtOAc (3x 30 mL) to yield compound **S17** (8.67 g, 81% mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 – 7.08 (m, 2H), 7.03 – 6.81 (m, 2H), 4.18 – 3.98 (m, 4H), 3.20 – 3.00 (m, 2H), 2.52 – 2.38 (m, 1H), 1.94 (d, J = 12.5 Hz, 2H), 1.83 – 1.66 (m, 2H), 1.29 – 1.07 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.59, 156.47, 120.45, 60.73, 46.33, 41.05, 27.65, 14.39. LC-MS (ESI): calcd for $C_{15}H_{19}N_3O_2$: 274.15500 $[M+H]^+$, found: 274.16 $[H]^+$, R_t = 5.75 min; HR-MS found 274.15523 $[M+H]^+$.

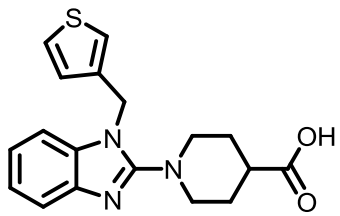


Ethyl 1-(1-benzyl-1H-benzo[d]imidazol-2-yl)piperidine-4-carboxylate (S18) The product was synthesized by procedure A starting from compound **S17** (140 mg, 0.38 mmol, 85%).^{1}H

NMR (400 MHz, DMSO-d_6) δ 7.46 – 6.96 (m, 9H), 5.25 (s, 2H), 4.15 – 3.99 (m, 2H), 3.41 (d, J = 12.3 Hz, 2H), 2.95 (t, J = 11.2 Hz, 2H), 2.60 – 2.50 (m, 1H), 1.93 – 1.62 (m, 4H), 1.16 (t, J = 6.9 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.73, 158.51, 141.99, 137.66, 135.78, 129.37, 128.02, 127.09, 121.93, 121.50, 118.02, 110.43, 60.61, 50.42, 47.53, 40.57, 28.09, 14.77. LC-MS (ESI): calcd for $C_{22}H_{25}N_3O$: 364.19 $[M+H]^+$, found: 364.22 $[H]^+$, R_t = 8.01 min.

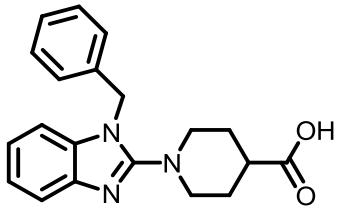


Ethyl 1-(1-(thiophen-3-ylmethyl)-1H-benzo[d]imidazol-2-yl)piperidine-4-carboxylate (S19) To a mixture of benzimidazole (2.0 g, 7.3 mmol), 3-hydroxymethyl thiophene (2.5 g, 22.0 mmol) and tri-*n*-butyl phosphine (5.4 mL, 22.0 mmol) in THF (20 mL) was added at 0°C TMAD (3.8 g, 22.0 mmol) in one portion. The resulting suspension was allowed to warm to room temperature and stirred at this temperature for 3 h. The reaction mixture was concentrated *in vacuo* and the residue was purified by column chromatography (petroleum ether : ethyl acetate 5:1 to 1:1) to afford compound **S19** (61%, 1.6 g). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (dt, J = 7.9, 1.0 Hz, 1H), 7.32 (dd, J = 5.0, 3.0 Hz, 1H), 7.21 – 7.16 (m, 1H), 7.12 – 7.09 (m, 2H), 7.02 (dd, J = 3.0, 1.3 Hz, 1H), 6.96 (dd, J = 5.0, 1.3 Hz, 1H), 5.18 (s, 2H), 4.16 (q, J = 7.1 Hz, 2H), 3.49 (dt, J = 6.7, 3.1 Hz, 2H), 3.02 (td, J = 12.5, 2.7 Hz, 2H), 2.48 (tt, J = 11.1, 4.1 Hz, 1H), 2.05 – 1.95 (m, 2H), 1.95 – 1.79 (m, 2H), 1.27 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.81, 158.38, 141.80, 137.71, 135.44, 127.28, 126.23, 122.15, 121.71, 121.70, 118.40, 109.49, 60.77, 50.73, 44.18, 41.10, 28.32, 14.45 LC-MS (ESI): calcd for $C_{20}H_{23}N_3O_2S$: 370.15837 $[M+H]^+$, found: 370.13 $[M+H]^+$, R_t = 6.60 min; HR-MS found 370.15890 $[M+H]^+$.



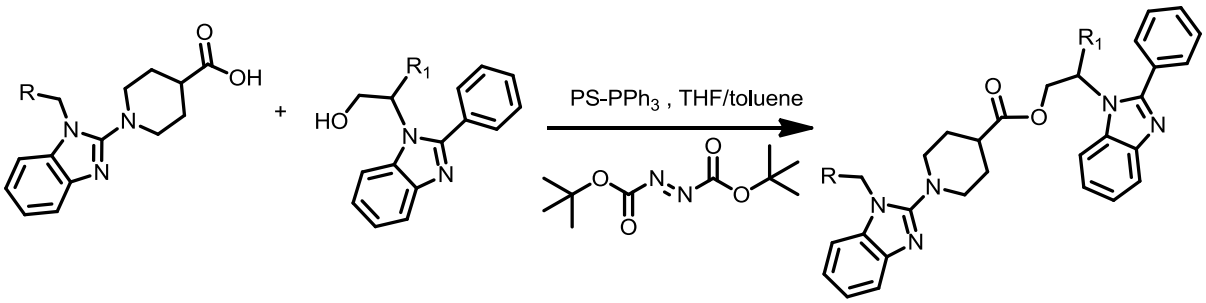
1-(1-(Thiophen-3-ylmethyl)-1H-benzo[d]imidazol-2-yl)piperidine-4-carboxylic acid (S20) To ester **S19** (1.6 g, 4.5 mmol) in MeOH/H_2O (40 mL, 10/1 vol/vol) was added KOH (1.3 g, 23 mmol) at room temperature. The resulting reaction mixture was stirred at room temperature for 5 h. The pH was adjusted with 20% acetic acid in H_2O to 6 and the aqueous

phase was extracted with CH_2Cl_2 (4 x 40 mL). The combined organic phases were dried over Na_2SO_4 and concentrated *in vacuo* to afford acid **S20** (89 %, 1.4 g). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 12.23 (s, 1H), 7.49 (dd, *J* = 5.0, 3.0 Hz, 1H), 7.40 (d, *J* = 7.1 Hz, 1H), 7.29 (s, 1H), 7.23 (d, *J* = 7.5 Hz, 1H), 7.08 – 7.00 (m, 2H), 6.94 (d, *J* = 4.9 Hz, 1H), 5.21 (s, 2H), 3.47 – 3.41 (m, 2H), 3.00 – 2.89 (m, 2H), 2.47 – 2.39 (m, 1H), 1.93 – 1.83 (m, 2H), 1.82 – 1.69 (m, 2H). ^{13}C NMR (151 MHz, DMSO) δ 176.50, 158.34, 138.47, 135.64, 127.85, 127.34, 125.99, 123.01, 121.88, 121.46, 117.96, 110.40, 50.59, 43.63, 28.22, 21.73. LC-MS (ESI): calcd for $C_{18}H_{19}N_3O_2S$: 342.12707 $[M+H]^+$, found: 342.10 $[M+H]^+$, R_t = 5.74 min; HR-MS found 342.12747 $[M+H]^+$.

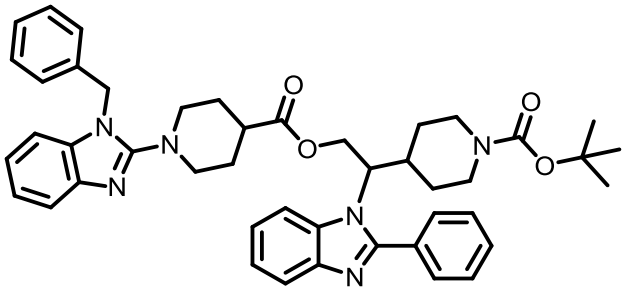


1-(1-Benzyl-1H-benzo[d]imidazol-2-yl)piperidine-4-carboxylic acid (S21) To ester **S18** (1.10 g, 3.0 mmol) in MeOH/H_2O (30 mL, 10/1 vol/vol) was added KOH (0.84 g, 15 mmol) at room temperature. The resulting reaction mixture was stirred at room temperature for 5 h. The pH was adjusted with 20% acetic acid in H_2O to 6 and the aqeous phase was extracted with CH_2Cl_2 (4x 30 mL). The combined organic phases were dried over Na_2SO_4,filtered and concentrated *in vacuo* to afford **S21** (92 %, 0.93 g). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.17 (s, 1H), 7.43 (d, *J* = 7.8 Hz, 1H), 7.31 (t, *J* = 7.6 Hz, 2H), 7.24 (t, *J* = 7.3 Hz, 1H), 7.16 (d, *J* = 7.5 Hz, 3H), 7.09 (t, *J* = 7.4 Hz, 1H), 7.03 (t, *J* = 7.5 Hz, 1H), 5.27 (s, 2H), 3.49 – 3.40 (m, 2H), 3.04 – 2.90 (m, 2H), 2.47 – 2.39 (m, 1H), 1.88 – 1.80 (m, 2H), 1.78 – 1.63 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.35, 172.61, 137.46, 135.50, 129.38, 128.06, 127.08, 122.17, 121.72, 117.56, 110.55, 50.42, 47.68, 28.12, 21.71. LC-MS (ESI): calcd for $C_{20}H_{21}N_3O_2$: 336.17065 $[M+H]^+$, found: 336.17 $[M+H]^+$, R_t = 5.82 min; HR-MS found 336.17101 $[M+H]^+$.

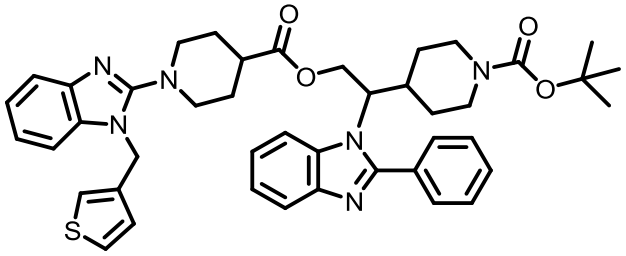
General Procedure (E) for esterification of two benzimidazole units



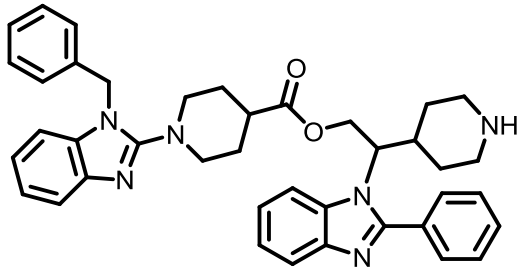
To a mixture of carboxylic acid, alcohol (1.0 eq) and polymer bound triphenylphosphine (3 meq) in THF/toluene (1:1, 3 mL/ mmol benzimidazole) was added at 0°C di-*tert*-butyl azodicarboylate (3eq). The reaction mixture is stirred at room temperature for 16 h, diluted with CH_2Cl_2 (15 mL/mmol benzimidazole) and filtered. The filtrate was concentrated *in vacuo* and purified by automated flash chromatography as described below.



tert-butyl 4-(2-(1-(1-benzyl-1H-benzo[d]imidazol-2-yl)piperidine-4-carbonyloxy)-1-(2-phenyl-1H-benzo[d]imidazol-1-yl)ethyl)piperidine-1-carboxylate (S22) The product was synthesized from alcohol **S10** (63 mg, 0.15 mmol) and carboxylic acid **S21** according to Procedure E for the esterification via Mitsunobu reaction. The crude product was purified by automated flash chromatography using a gradient of cyclohexane and ethyl acetate 20:1 to 1:2 to yield compound **S22** (70 mg, 0.10 mmol, 64%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, *J* = 7.6 Hz, 1H), 7.63 – 7.50 (m, 3H), 7.50 – 7.38 (m, 4H), 7.31 – 7.16 (m, 5H), 7.15 – 6.98 (m, 4H), 6.95 (d, *J* = 7.8 Hz, 1H), 5.08 (s, 2H), 4.75 (t, *J* = 10.2 Hz, 1H), 4.51 – 4.30 (m, 2H), 4.14 – 3.96 (m, 1H), 3.95 – 3.69 (m, 1H), 3.41 – 3.14 (m, 2H), 2.97 – 2.72 (m, 2H), 2.63 (t, *J* = 12.0 Hz, 1H), 2.51 – 2.14 (m, 3H), 1.84 – 1.41 (m, 4H), 1.39 – 1.04 (m, 10H), 1.04 – 0.63 (m, 3H).^{13}C NMR (101 MHz, $CDCl_3$) δ 174.16, 158.09, 155.58, 154.75, 143.80, 136.34, 135.40, 133.42, 130.67, 130.15, 130.10, 129.23, 128.99, 127.93, 126.24, 123.18, 122.92, 122.34, 121.91, 120.87, 118.24, 111.79, 109.67, 79.97, 63.33, 60.90, 50.50, 50.35, 47.86, 40.80, 36.99, 30.13, 28.82, 28.60, 27.92, 27.88. LC-MS (ESI): calcd for $C_{45}H_{50}N_6O_4$: 739.39663 $[M+H]^+$, found: 739.39 $[M+H]^+$, R_t = 7.38 min; HR-MS found 739.39738 $[M+H]^+$. Chiral HPLC conditions: flow rate: 0.35 mL/min; solvent: 75 % ethanol in *iso*-hexane. R_T: (*R*)-26.5 min, (*S*)-56.8 min.

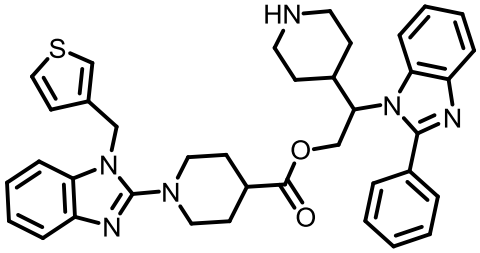


tert-Butyl 4-(1-(2-phenyl-1H-benzo[d]imidazol-1-yl)-2-(1-(1-(thiophen-3-ylmethyl)-1H-benzo[d]imidazol-2-yl)piperidine-4-carbonyloxy)ethyl)piperidine-1-carboxylate (S23) The product was synthesized from alcohol **S10** (80 mg, 0.19 mmol) and carboxylic acid **S20** according to Procedure E for the esterification via Mitsunobu reaction. The crude product was purified by automated flash chromatography using a gradient of cyclohexane and ethyl acetate 20:1 to 1:2 to yield compound **S23** (95 mg, 0.13 mmol, 67%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (d, *J* = 7.7 Hz, 1H), 7.66 (s, 2H), 7.59 (t, *J* = 8.4 Hz, 1H), 7.57 – 7.45 (m, 4H), 7.36 – 7.22 (m, 3H), 7.23 – 7.14 (m, 1H), 7.14 – 7.05 (m, 2H), 6.97 (d, *J* = 1.6 Hz, 1H), 6.91 (d, *J* = 5.0 Hz, 1H), 5.11 (s, 2H), 4.83 (t, *J* = 10.0 Hz, 1H), 4.59 – 4.42 (m, 2H), 4.15 (d, *J* = 11.3 Hz, 1H), 4.03 – 3.86 (m, 1H), 3.50 – 3.23 (m, 2H), 3.02 – 2.79 (m, 2H), 2.78 – 2.62 (m, 1H), 2.54 – 2.25 (m, 3H), 1.85 (d, *J* = 11.8 Hz, 1H), 1.79 – 1.53 (m, 4H), 1.40 (s, 9H), 1.23 (d, *J* = 11.9 Hz, 1H), 1.00 (d, *J* = 26.4 Hz, 1H), 0.92 (d, *J* = 9.8 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.21, 158.07, 155.60, 154.75, 143.82, 141.66, 137.58, 135.36, 133.42, 130.69, 130.16, 130.12, 129.00, 127.32, 126.20, 123.18, 122.93, 122.20, 121.79, 121.72, 120.88, 118.39, 111.79, 109.50, 79.98, 63.31, 60.90, 50.53, 50.36, 44.10, 40.88, 37.01, 30.13, 28.83, 28.60, 27.97, 27.93. LC-MS (ESI): calcd for $C_{43}H_{48}N_6O_4S$: 745.35305 $[M+H]^+$, found: 745.33 $[M+H]^+$, R_t = 7.35 min; HR-MS found 745.35302 $[M+H]^+$. Chiral HPLC conditions: flow rate: 0.35 mL/min; solvent: 75 % ethanol in *iso*-hexane. R_T: (*R*)-26.8 min, (*S*)-54.6 min.



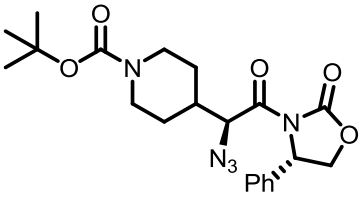
2-(2-Phenyl-1H-benzo[d]imidazol-1-yl)-2-(piperidin-4-yl)ethyl 1-(1-benzyl-1H-benzo[d]imidazol-2-yl)piperidine-4-carboxylate (5) The product was synthesized according to General Procedure (D) starting from the protected piperidine **S22** (15 mg,0.02 mmol) to afford the HCl-salt in quantitative yield. The residue was dissolved in methanol (500 µL) and applied to a preparative C18-RP column eluting with a gradient (CH_3CN: H_2O 1:5 to 9:1 containing 0.1% TFA), to afford piperidine **11** (9 mg, 0.01 mmol, 71%) as a TFA-salt. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.08 (s, 1H), 8.78 (s, 1H), 8.07 (s, 1H), 7.81 – 7.72 (m, 3H), 7.72 – 7.59 (m, 3H), 7.56 (d, *J* = 7.8 Hz, 1H), 7.43 (s, 2H), 7.39 – 7.30 (m, 5H), 7.28 (t, *J* = 7.7 Hz, 1H), 7.24 (d, *J* = 7.5 Hz, 2H), 5.39 (s, 2H), 4.83 (t, *J* = 10.4 Hz, 1H), 4.57 (d, *J* = 11.3 Hz, 1H), 4.54 – 4.43 (m, 1H), 3.65 (d, *J* = 12.1 Hz, 1H), 3.57 (d, *J* = 11.9 Hz, 1H), 3.33 – 3.17 (m, 3H), 3.04 (d, *J* = 11.3 Hz, 1H), 2.85 – 2.72 (m, 2H), 2.72 – 2.54 (m, 2H), 1.97 (d, *J* = 12.3

Hz, 1H), 1.69 – 1.58 (m, 2H), 1.58 – 1.44 (m, 2H), 1.40 (d, *J* = 10.8 Hz, 1H), 1.28 – 1.07 (m,2H). LC-MS (ESI): calcd for $C_{40}H_{42}N_6O_2$: 639.34420 $[M+H]^+$, found: 639.36 $[M+H]^+$, R_t = 5.41 min; HR-MS found 639.34491 $[M+H]^+$.

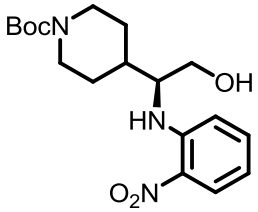


2-(2-Phenyl-1H-benzo[d]imidazol-1-yl)-2-(piperidin-4-yl)ethyl 1-(1-(thiophen-3-ylmethyl)-1H-benzo[d]imidazol-2-yl)piperidine-4-carboxylate (6) The product was synthesized according to General Procedure (D) starting from the protected piperidine (61 mg, 0.08 mmol) to afford the HCl-salt in quantitative yield. The residue was dissolved in methanol (500 µL) and applied to a preparative C18-RP column eluting with a gradient (CH_3CN: H_2O 1:5 to 9:1 containing 0.1% acetic acid), to afford free piperidine **6** (33 mg, 0.005 mmol, 59%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.84 (d, *J* = 6.0 Hz, 1H), 7.69 – 7.62 (m, 3H), 7.58 – 7.50 (m, 3H), 7.51 – 7.46 (m, 1H), 7.39 (t, *J* = 8.0 Hz, 1H), 7.26 – 7.17 (m, 4H), 7.08 – 6.99 (m, 2H), 6.92 – 6.87 (m, 1H), 5.15 (s, 2H), 4.89 – 4.77 (m, 1H), 4.54 (dd, *J* = 12.0, 3.5 Hz, 1H), 4.48 – 4.34 (m, 1H), 3.32 (d, *J* = 12.6 Hz, 1H), 3.24 (d, *J* = 12.5 Hz, 1H), 3.01 – 2.78 (m, 4H), 2.72 (d, *J* = 11.8 Hz, 1H), 2.45 – 2.36 (m, 1H), 2.26 – 2.18 (m, 1H), 1.80 – 1.76 (m, 1H), 1.64 – 1.38 (m, 4H), 1.14 – 1.05 (m, 1H), 0.86 – 0.70 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 174.30, 158.13, 155.48, 143.70, 141.96, 138.43, 135.64, 133.83, 131.19, 130.50, 130.37, 129.35, 127.87, 127.28, 123.22, 122.93, 122.78, 121.88, 121.47, 120.28, 117.96, 113.16, 110.36, 109.99, 63.22, 61.45, 50.12, 45.73, 45.62, 43.61, 36.18, 29.43, 27.81, 27.68. LC-MS (ESI): calcd for $C_{38}H_{40}N_6O_2S$: 645.30062 $[M+H]^+$, found: 645.27 $[M+H]^+$, R_t = 5.31 min; HR-MS found 645.30123 $[M+H]^+$.

Enantioselective synthesis of PDEδ binding small molecules

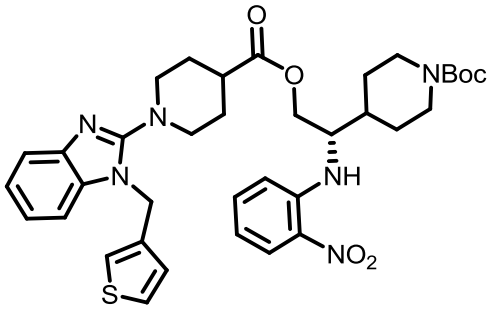


tert-Butyl 4-((S)-1-azido-2-oxo-2-((S)-2-oxo-4-phenyloxazolidin-3-yl)ethyl)piperidine-1-carboxylate (S24) The product was synthesized according to literature methods.[1] Analytical data are consistent with literature data.



(S)-tert-Butyl 4-(2-hydroxy-1-(2-nitrophenylamino)ethyl)piperidine-1-carboxylate (S25) To a solution of azide **S24** (2.0 g, 4.7 mmol) in MeOH (5 mL) was added $NaBH_4$ (530 mg, 14.1 mmol) at room temperature. The reaction mixture was stirred at room temperature for 2 h and $NaBH_4$ (530 mg, 14.1 mmol) was added to the reaction mixture. After stirring for an additional 12 h sat. NH_4Cl-solution (20 mL) was added, followed by CH_2Cl_2 (30 mL). The aqueous layer was extracted with CH_2Cl_2 (3x 30 mL). The combined organic layers were dried (Na_2SO_4), filtered, evaporated to dryness and the residue was purified by flash chromatography (gradient cyclohexane : ethyl acetate 10:1 to 1:1). To a solution of the intermediate azido alcohol (1.0 g, 3.67 mmol) in degassed ethanol was added palladium on carbon (5%, 100 mg). The reaction vessel was evacuated and then refilled with argon three times. The reaction mixture was stirred under H_2 (1 atm) for 2 h at room temperature. The reaction vessel was then evacuated and refilled with argon, ethyl acetate (50 mL) was added and filtered over Celite. The filter cake was washed with ethanol and ethyl acetate (100 mL, respectively), evaporated to dryness and used without any further purification (LC-MS:ESI 244.89 $[M+H]^+$). To a solution of the free amine in NMP (10 mL) was added 1-fluoro-2-nitrobenzene (0.66 mL, 6.14 mmol), and DIPEA (0.35 mL, 2 mmol). The reaction mixture was heated to 90°C for 8 h, cooled to room temperature and diluted with CH_2Cl_2 (50 mL). To the reaction mixture was added a sat. of NH_4Cl solution (40 mL). The aqueous layer was extracted with CH_2Cl_2 (2x 50 mL). The combined organic layers were dried over

$MgSO_4$,filtered and then evaporated to dryness. The crude product was purified by column chromatography to yield compound **S25** (1.35 g, 3.7 mmol, 45%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (d, *J* = 9.1 Hz, 1H), 8.09 (dd, *J* = 8.6, 1.5 Hz, 1H), 7.33 (dd, *J* = 8.4, 7.2 Hz, 1H), 6.85 (d, *J* = 8.8 Hz, 1H), 6.57 (dd, *J* = 8.4, 7.2 Hz, 1H), 4.19 – 3.97 (m, 2H), 3.77 (dd, *J* = 11.2, 4.2 Hz, 1H), 3.70 (dd, *J* = 11.1, 5.3 Hz, 1H), 3.59 – 3.48 (m, 1H), 2.61 (t, *J* = 12.3 Hz, 2H), 2.00-1.96 (m, 1H), 1.87 – 1.57 (m, 4H), 1.37 (s, 9H), 1.31 – 1.10 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.92, 145.89, 136.52, 132.42, 127.38, 115.81, 114.42, 79.82, 62.56, 58.73, 38.44, 29.21, 28.74, 28.64. LC-MS (ESI): calcd for $C_{18}H_{27}N_3O_5$: 388.18429 $[M+Na]^+$, R_t = 9.41 min; HR-MS found 388.18459 $[M+Na]^+$; $[\alpha]_D^{20}$: -243.0° (c=1.6, CH_2Cl_2).

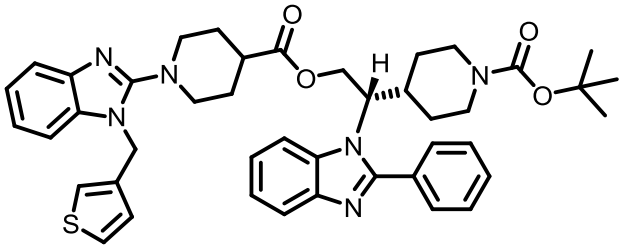


(S)-tert-Butyl 4-(1-(2-nitrophenylamino)-2-(1-(1-(thiophen-3-ylmethyl)-1H-benzo[d]imidazol-2-yl)piperidine-4-carbonyloxy)ethyl)piperidine-1-carboxylate (S26)

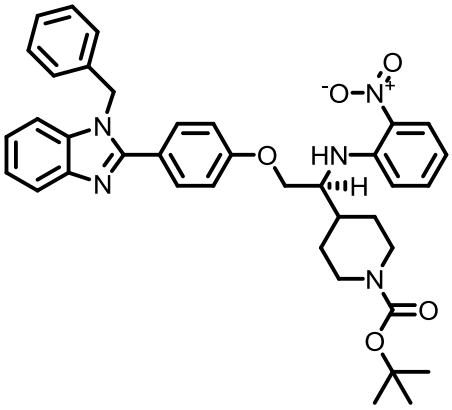
The product was synthesized according to Procedure D for the connection of two benzimidazoles via Mitsunobu reaction starting from alcohol **S25** (1.0 g. 2.74 mmol). The crude product was purified by automated flash chromatography using a gradient of cyclohexane: ethyl acetate 20:1 to 1:2 to yield compound **S26** (1.33 g, 1.94 mmol, 71%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 – 8.13 (m, 2H), 7.63 – 7.58 (m, 1H), 7.43 – 7.38 (m, 1H), 7.33 (dd, *J* = 5.0, 3.0 Hz, 1H), 7.22 – 7.16 (m, 1H), 7.13 – 7.10 (m, 2H), 7.04 – 6.99 (m, 1H), 6.97 – 6.89 (m, 2H), 6.64 (ddd, *J* = 8.1, 7.0, 1.1 Hz, 1H), 5.16 (s, 2H), 4.30 – 4.08 (m, 4H), 3.79 (td, *J* = 11.5, 5.5 Hz, 1H), 3.45 (d, *J* = 12.5 Hz, 2H), 2.96 (dd, *J* = 16.8, 7.0 Hz, 2H), 2.70 (t, *J* = 12.1 Hz, 2H), 2.47 (tt, *J* = 11.2, 4.1 Hz, 1H), 1.98 – 1.69 (m, 6H), 1.37 – 1.17 (m, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 174.55, 158.15, 154.85, 145.39, 141.69, 137.65, 136.53, 135.40, 132.60, 127.42, 127.32, 126.25, 122.20, 121.78, 121.74, 118.37, 116.08, 114.18, 109.52, 79.91, 64.16, 55.71, 50.60, 50.53, 44.17, 41.00, 38.96, 29.11, 28.65, 28.13. LC-MS (ESI): calcd for $C_{18}H_{27}N_3O_5$: 689.31158 $[M+H]^+$, found: 689.29 $[M+H]^+$, R_t = 8.47 min; HR-MS found 689.31133 $[M+H]^+$; $[\alpha]_D^{20}$: -50.0° (c=1.8, CH_2Cl_2).

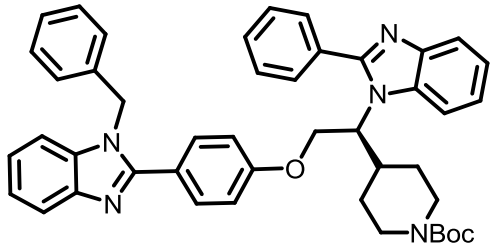


(S)-tert-Butyl 4-(1-(2-phenyl-1H-benzo[d]imidazol-1-yl)-2-(1-(1-(thiophen-3-ylmethyl)-1H-benzo[d]imidazol-2-yl)piperidine-4-carbonyloxy)ethyl)piperidine-1-carboxylate (***S***)-**S23**) To a solution of **S26** (30 mg, 0,044 mmol) in MeOH, H_2O and DMSO (8:2:1, 3 mL) was added benzaldehyde (9µL, 0.09 mmol) and sodium dithionite (23 mg, 0.14 mmol). The reaction mixture was heated to 65°C and after 0.5, 1, and 2 h sodium dithionite (3x 23 mg, 0.14 mmol) was added. After 5 h the reaction mixture was allowed to cool to room temperature. The reaction mixture was concentrated under reduced pressure and then extracted with CH_2Cl_2 (3x 20 mL). The combined organic phases were dried (Na_2SO_4), filtered and concentrated to dryness. Purification by flash chromatography (gradient cyclohexane : ethyl acetate 5:1 to 1:5) afforded enantiomerically pure (*S*)-**S23** (20 mg, 0.031mmol, 61%).

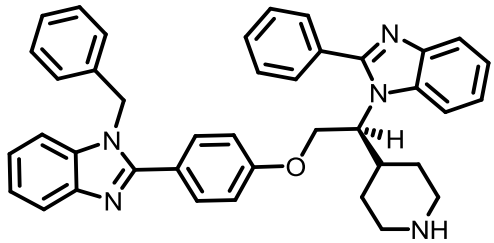


(*S*)-tert-Butyl 4-(2-(4-(1-benzyl-1H-benzo[d]imidazol-2-yl)phenoxy)-1-(2-nitrophenylamino)ethyl)piperidine-1-carboxylate (S27) The product was synthesized according to Procedure C for the connection of two benzimidazoles via Mitsonobu reaction starting from alcohol **S25** (30 mg. 0.10 mmol) The crude product was purified by automated flash chromatography using a gradient of of cyclohexane: ethyl acetate 20:1 to 1:2 to yield compound **S27** (45 mg, 0.07 mmol, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36 (d, *J* = 9.1 Hz, 1H), 8.17 (dd, *J* = 8.6, 1.6 Hz, 1H), 7.83 (t, *J* = 7.3 Hz, 1H), 7.63 – 7.58 (m, 2H), 7.45 – 7.39 (m, 1H), 7.36 – 7.26 (m, 4H), 7.24 – 7.15 (m, 2H), 7.13 – 7.07 (m, 2H), 6.97 – 6.89 (m, 3H), 6.70 – 6.62 (m, 1H), 5.42 (s, 2H), 4.35 – 4.01 (m, 3H), 3.97 – 3.77 (m, 1H), 2.72 (t, *J* = 12.0 Hz, 2H), 2.10 – 1.99 (m, 1H), 1.90 (d, *J* = 12.6 Hz, 1H), 1.77 (d, *J* = 12.7 Hz, 1H), 1.49 – 1.21

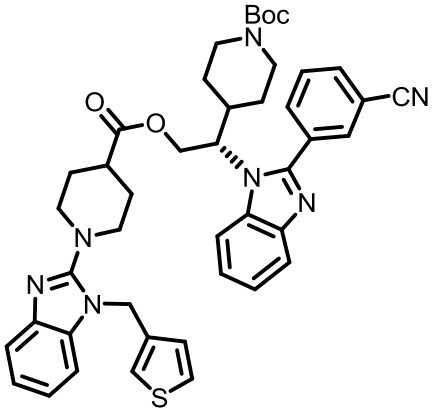
(m, 12H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.86, 154.88, 154.08, 145.31, 143.28, 136.66, 136.51, 136.32, 132.56, 131.01, 129.29, 127.98, 127.45, 126.10, 123.34, 123.12, 122.87, 119.96, 115.91, 115.04, 114.10, 110.60, 79.84, 67.75, 56.27, 48.58, 38.80, 29.21, 28.70, 28.65. $[\alpha]_D^{20}$: -62.5° (c=1.5, CH_2Cl_2).



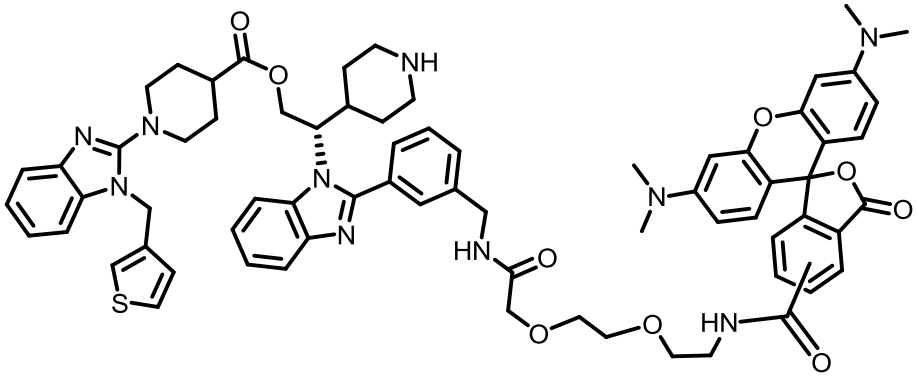
(*S*)-tert-Butyl 4-(2-(4-(1-benzyl-1H-benzo[d]imidazol-2-yl)phenoxy)-1-(2-phenyl-1H-benzo[d]imidazol-1-yl)ethyl)piperidine-1-carboxylate (*S*)-(S16) To a solution of **S27** (40 mg, 0.066 mmol) in MeOH, H_2O and DMSO (8:2:1, 2 mL) was added benzaldehyde (18 µL, 0.18 mmol) and sodium dithionite (23 mg, 0.14 mmol). The reaction mixture was heated to 65°C and after 0.5, 1, and 2 h sodium dithionite (3x 23 mg, 0.14 mmol) was added. After 6 h the reaction mixture was allowed to cool to room temperature. The reaction mixture was concentrated under reduced pressure and then extracted with CH_2Cl_2 (3x 20 mL). The combined organic phases were dried (Na_2SO_4), filtered and concentrated to dryness. Purification by flash chromatography (gradient cyclohexane : ethyl acetate 5:1 to 1:5) afforded enantiomerically pure (*S*)-(**S16**) (25 mg, 0.035mmol, 53%).



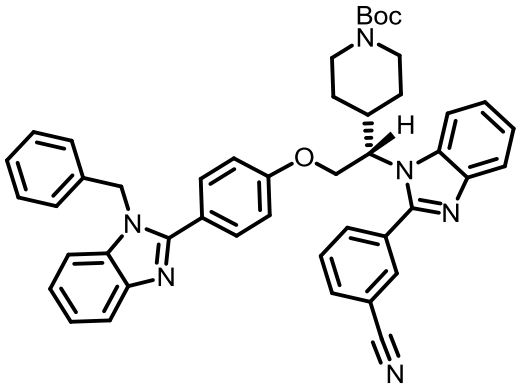
(S)-1-benzyl-2-(4-(2-(2-phenyl-1H-benzo[d]imidazol-1-yl)-2-(piperidin-4-yl)ethoxy)phenyl)-1H-benzo[d]imidazole Deltarasin (*S*)-4 The product was synthesized according to General Procedure (D) starting from (*S*)-**S16** (16 mg, 0.023 mmol) to afford the HCl-salt in quantitative yield. The residue was dissolved in methanol (400 µL) and applied to a preparative C18-RP column eluting with a gradient (CH_3CN: H_2O 1:5 to 9:1 containing 0.1% TFA), to afford the desired piperdine (10 mg, 0.0165 mmol, 71%) as a TFA-salt. For xenograft experiments the compound was purified via manual reverse phase chromatography and used as an HCl-salt.



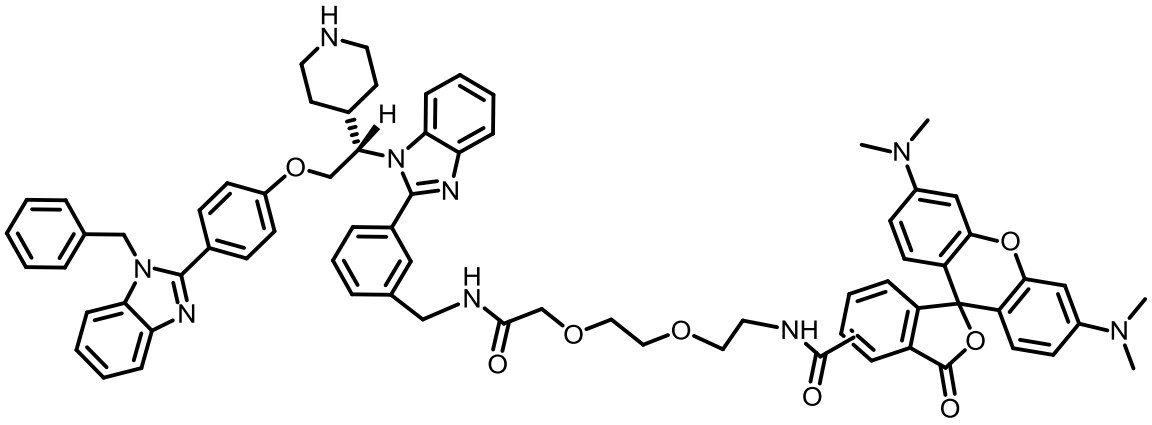
(*S*)-tert-Butyl 4-(1-(2-(3-cyanophenyl)-1H-benzo[d]imidazol-1-yl)-2-(1-(1-(thiophen-3-ylmethyl)-1H-benzo[d]imidazol-2-yl)piperidine-4-carbonyloxy)ethyl)piperidine-1-carboxylate (S28) To a solution of **S26** (100 mg, 0.15 mmol) in MeOH, H_2O and DMSO (8:2:1, 5 mL) was added 3-cyano benzaldehyde (22 mg, 0.17 mmol) and sodium dithionite (81 mg, 0.46 mmol). The reaction mixture was heated to 65°C and after 0.5, 1, and 2 h sodium dithionite (3x 81 mg, 0.46 mmol) was added. After 8 h the reaction mixture was allowed to cool to room temperature. The reaction mixture was stirred then under air for 3 days. The reaction mixture was concentrated under reduced pressure and then extracted with CH_2Cl_2 (3x 50 mL). The combined organic phases were dried (Na_2SO_4), filtered and concentrated to dryness. Purification by flash chromatography (gradient cyclohexane : ethyl acetate 5:1 to 1:5) afforded **S28** (74 mg, 0.010 mmol, 64%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 – 7.95 (m, 2H), 7.87 (dd, *J* = 12.8, 5.8 Hz, 2H), 7.74 (t, *J* = 7.4 Hz, 2H), 7.60 (d, *J* = 5.1 Hz, 1H), 7.46 – 7.27 (m, 5H), 7.17 (d, *J* = 7.9 Hz, 1H), 7.08 (d, *J* = 1.4 Hz, 1H), 6.93 (d, *J* = 5.0 Hz, 1H), 5.20 (s, 2H), 5.04 – 4.91 (m, 1H), 4.62 (dd, *J* = 11.9, 3.8 Hz, 1H), 4.38 (t, *J* = 7.9 Hz, 1H), 4.17 (d, *J* = 13.3 Hz, 1H), 4.00 – 3.83 (m, 1H), 3.77 – 3.56 (m, 2H), 3.42 – 3.22 (m, 2H), 2.70 (t, *J* = 12.3 Hz, 1H), 2.62 – 2.34 (m, 3H), 2.00 – 1.79 (m, 3H), 1.79 – 1.53 (m, 2H), 1.40 (s, 9H), 1.24 (t, *J* = 10.1 Hz, 1H), 0.96 – 0.67 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.09, 161.89, 161.53, 154.66, 153.12, 152.33, 141.54, 134.45, 134.10, 133.52, 132.74, 132.02, 130.82, 130.63, 130.54, 128.67, 125.59, 125.53, 124.92, 124.74, 124.36, 122.56, 120.44, 118.01, 117.93, 115.05, 113.60, 112.28, 110.59, 80.21, 63.52, 61.71, 49.35, 46.05, 39.68, 37.35, 29.93, 28.83, 28.57, 27.50. LC-MS (ESI): calcd for $C_{18}H_{27}N_3O_5$: 689.31158 $[M+H]^+$, found: 689.29 $[M+H]^+$, R_t = 8.47 min; HR-MS found 689.31133 $[M+H]^+$.



TAMRA-(S)-2-(2-(3-(3,12-dioxo-5,8-dioxa-2,11-diazatridecyl)phenyl)-1H-enzo[d]imidazol-1-yl)-2-(piperidin-4-yl)ethyl 1-(1-(thiophen-3-ylmethyl)-1H-benzo[d]imidazol-2-yl)piperidine-4-carboxylate = TAMRA-(*S*)-6 Raney Nickel (Aldrich, W.R. Grace and Co. Raney®2800, slurry, in H_2O, 40 mg) was added to an argon filled flask containing ammonia in ethanol (2.0 M, 1 mL). After 5 minutes of stirring the liquid was removed via syringe. The flask was evacuated and flushed with argon. Ammonia in ethanol (2.0 M, 4 mL) was then added, followed by compound **S28** (15 mg, 19 µmol). The solution was purged with argon for 2 minutes and kept under an atmosphere of hydrogen (1 atm, balloon). The resulting reaction mixture was stirred at room temperature for 3 h to afford the free amine (LC-MS: 6.36 min retention time, $[M+H]^+$ 774.36). The reaction mixture was filtered over a pad of Celite, the filter cake was washed with EtOH (30 mL) and ethylacetate (20 ml) and the filtrate was concentrated under reduced pressure. To the crude amine in DMF (1 mL) was added PyBOP (12 mg, 23 µmol),TAMRA-PEG-COOH (13 mg, 23 µmol) and triethylamine (6,5 µL). The reaction mixture was stirred at room temperature overnight. The reaction mixture was concentrated under reduced pressure. To the residue was added HCl in dioxane (4 M) and the mixture was stirred for 2 h at room temperature. The reaction mixture was concentrated under reduced pressure, the residue was dissolved in methanol and purified by reverse phase chromatography to yield TAMRA-(*S*)-**6** (7 mg, 6 µmol, 29 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.95 (t, J = 5.5 Hz, 1H), 8.63 (s, 1H), 8.54 – 8.46 (m, 1H), 8.46 – 8.38 (m, 1H), 8.30 – 8.23 (m, J = 8.1 Hz, 1H), 8.11 – 7.98 (m, 1H), 7.84 (d, J = 7.6 Hz, 1H),7.67 – 7.60 (m, 2H), 7.58 – 7.39 (m, 6H), 7.35 – 7.22 (m, 4H), 7.22 – 7.11 (m, 2H), 7.06 – 6.87 (m, 6H), 5.23 (s, 2H), 4.89 (s, 1H), 4.56-4.38 (m, J = 45.4, 9.5 Hz, 3H), 3.97 (s, 2H), 3.08 – 2.90 (m, 3H), 2.88 – 2.76 (m, 1H), 2.75 – 2.61 (m, 1H) 2.08 – 1.91 (m, 2H), 1.71 – 1.27 (m, 7H), 1.27 – 1.13 (m, 2H), 1.15 – 0.91 (m, 3H). LC-MS (ESI): found: 1231.58 $[M+H]^+$, R_t = 5.76 min; HR-MS calcd for $C_{70}H_{74}N_{10}O_9S$: 411.18597 $[M+3H]^{3+}$, found 411.18576 $[M+3H]^{3+}$.

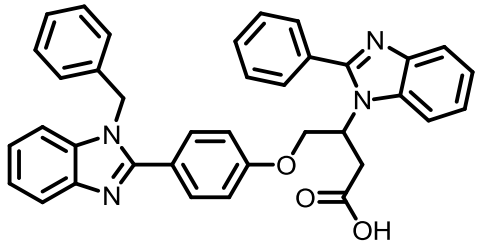


(*S*)-3-(1-(2-(4-(1-benzyl-1H-benzo[d]imidazol-2-yl)phenoxy)-1-(piperidin-4-yl)ethyl)-1H-benzo[d]imidazol-2-yl)benzonitrile (*S*)-S29 The desired product was synthesized according to the procedure for **S28** starting from **S27** (yield 60%).^{1}H NMR (400 MHz, cdcl$_3$) δ 8.09 (s, 1H), 7.98 (d, *J* = 7.7 Hz, 1H), 7.80 – 7.71 (m, 3H), 7.63 – 7.51 (m, 4H), 7.31 – 7.20 (m, 6H), 7.18 – 7.10 (m, 2H), 7.03 – 6.97 (m, 2H), 6.85 (d, *J* = 8.8 Hz, 2H), 5.33 (s, 2H), 4.72 (t, *J* = 9.2 Hz, 1H), 4.49 – 4.34 (m, 2H), 4.17 – 4.02 (m, 1H), 3.92 – 3.72 (m, 1H), 2.64 (t, *J* = 12.5 Hz, 1H), 2.56 – 2.31 (m, 2H), 1.85 (d, *J* = 12.9 Hz, 1H), 1.33 (s, 9H), 1.26 – 1.11 (m, 1H), 0.86 – 0.74 (m, 2H). LC-MS (ESI): calcd for $C_{46}H_{45}N_6O_3$ 729.25 $[M+H]^+$, found: 729.31 $[M+H]^+$.



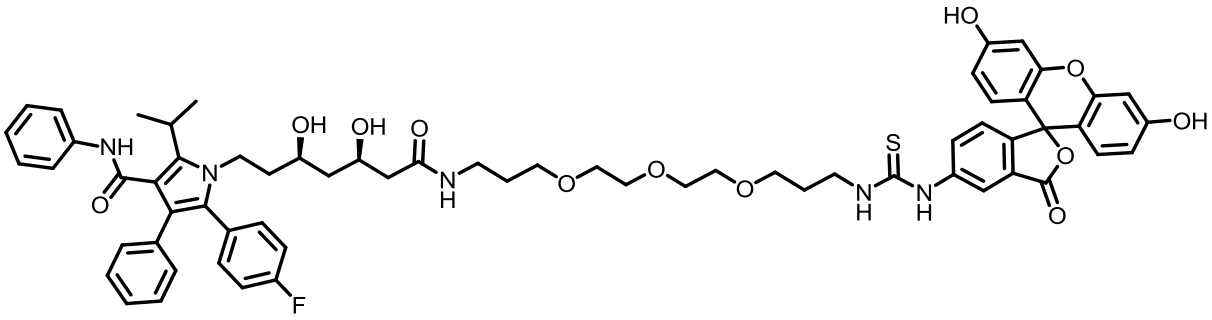
(*S*)-N-(2-(2-(2-(3-(1-(2-(4-(1-benzyl-1H-benzo[d]imidazol-2-yl)phenoxy)-1-(piperidin-4-yl)ethyl)-1H-benzo[d]imidazol-2-yl)benzylamino)-2-oxoethoxy)ethoxy)ethyl)-3',6'-bis(dimethylamino)-3-oxo-3H-spiro[isobenzofuran-1,9'-xanthene]-5-carboxamide TAMRA-(*S*)-4 The deisred product was synthesized according to the procedure for TAMRA-(*S*)-**6** starting from (*S*)-**S29**. (yield 20%).^{1}H NMR (400 MHz, dmso) δ 8.94 (t, *J* = 5.5 Hz, 1H), 8.70 – 8.58 (m, 2H), 8.40 (t, *J* = 6.2 Hz, 1H), 8.24 (dd, *J* = 8.0, 1.7 Hz, 1H), 8.19 – 8.02 (m, 1H), 7.95 (d, *J* = 7.0 Hz, 1H), 7.78 – 7.57 (m, 6H), 7.57 – 7.41 (m, 4H), 7.41 – 7.19 (m, 6H), 7.15 – 6.87 (m, 9H), 5.55 (s, 2H), 4.86 (t, *J* = 9.6 Hz, 1H), 4.67 – 4.48 (m, 2H), 4.43 (d, *J* = 6.0 Hz, 2H), 3.94 (s, 2H), 2.79 – 2.63 (m, 2H), 2.10 (d, *J* = 12.5 Hz, 1H), 1.50 – 1.34 (m, 1H),

1.11 – 0.87 (m, 2H). LC-MS (ESI): calcd for $C_{72}H_{71}N_9O_8$: 1190.54 $[M+H]^+$, found: 1190.1 $[M+H]^+$.



4-(4-(1-Benzyl-1H-benzo[d]imidazol-2-yl)phenoxy)-3-(2-phenyl-1H-benzo[d]imidazol-1-yl)butanoic acid (S30) To a suspension of **2** (20 mg, 36 µmol) in a mixture of CCl_4, acetonitrile and water 1:1:1.5 (2 mL) was added sodium periodate (31 mg, 140 µM) and $RuCl_3$ (0.3 mg). After 24h of stirring, sodium periodate (31 mg, 140 µM) and $RuCl_3$ (0.3 mg) were added. The reaction mixture was stirred for an additional 2 d. The reaction mixture was concentrated *in vacuo*. The aqueous residue was extracted with CH_2Cl_2 (3x10 mL). The combined organic phases were dried (Na_2SO_4), filtered and concentrated *in vacuo*. The crude reaction mixture was subjected to automated flash chromatography (gradient CH_2Cl_2: MeOH 100:1 to 15:1) to afford the desired compound (13 mg, 22 µmol, 62%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.91 (d, *J* = 7.1 Hz, 1H), 7.76 (dd, *J* = 7.2, 2.0 Hz, 2H), 7.65 (dd, *J* = 10.7, 4.8 Hz, 2H), 7.56 (d, *J* = 8.7 Hz, 2H), 7.48 (dd, *J* = 8.2, 2.8 Hz, 3H), 7.39 (d, *J* = 6.4 Hz, 1H), 7.32 – 7.14 (m, 7H), 6.96 (t, *J* = 8.0 Hz, 4H), 5.50 (s, 2H), 5.23 (s, 1H), 4.71 (t, *J* = 9.9 Hz, 1H), 4.39 (dd, *J* = 10.7, 4.0 Hz, 1H), 3.25 – 3.14 (m, 2H).^{13}C NMR (101 MHz, DMSO-d_6) δ 159.42, 155.70, 153.73, 143.95, 143.35, 137.67, 136.57, 133.90, 131.60, 131.12, 130.59, 130.07, 129.47, 128.95, 128.11, 126.68, 123.49, 123.11, 122.77, 122.60, 120.24, 119.72, 115.25, 113.18, 111.60, 68.23, 53.52, 48.10, 34.96, 29.68. LC-MS (ESI): calcd for $C_{37}H_{30}N_4O_3$: 579.23907 $[M+H]^+$, found: 579.36 $[M+H]^+$, R_t = 6.26 min; HR-MS found 579.23912 $[M+H]^+$.

Procedure for labeling of atorvastatin with fluorescein



1-((19R,21R)-1-(3',6'-Dihydroxy-3-oxo-3H-spiro[isobenzofuran-1,9'-xanthene]-5-ylamino)-19,21-dihydroxy-17-oxo-1-thioxo-6,9,12-trioxa-2,16-diazatricosan-23-yl)-5-(4-fluorophenyl)-2-isopropyl-N,4-diphenyl-1H-pyrrole-3-carboxamide (S31) To Atorvastatin (20 mg, 35 µmol) in DMF (200 µL) was added DIPEA (24 µL, 140 µmol), FITC-PEG_3-NH_2[10]_ (90 mg, 148 µmol) and PyBOP (27 mg, 50 µmol). The reaction mixture was stirred for 36 hours and then directly applied to a reverse phase C-18 column (gradient water: acetontrile 9:1 to 1:5 containing 0.1% TFA) to yield fluorescein-labeled atorvastatin (17 mg, 15 µmol, 41%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.89 (s, 1H), 9.77 (s, 1H), 8.19 (s, 1H), 8.05 (s, 1H), 7.75 (t, J = 5.6 Hz, 1H), 7.69 (s, 1H), 7.47 (d, J = 8.0 Hz, 2H), 7.24 – 7.13 (m, 8H), 7.07 – 7.01 (m, 4H), 6.99 – 6.92 (m, 2H), 6.66 – 6.63 (m, 2H), 6.59 – 6.52 (m, 4H), 3.94 – 3.87 (m, 1H), 3.79 (dd, J = 11.9, 7.1 Hz, 1H), 3.75 – 3.68 (m, 2H), 3.24 – 3.15 (m, 2H), 3.10 – 2.98 (m, 3H), 2.09 (d, J = 6.3 Hz, 2H), 1.78 (p, J = 6.5 Hz, 2H), 1.64 – 1.53 (m, 4H), 1.53 – 1.43 (m, 1H), 1.41 – 1.30 (m, 8H), 1.28 – 1.17 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 171.26, 169.20, 166.81, 161.35, 160.14, 152.53, 136.57, 135.55, 129.79, 129.68, 129.32, 129.11, 128.30, 127.93, 126.03, 123.65, 121.22, 120.04, 118.06, 116.11, 115.96, 113.25, 110.37, 102.88, 70.42, 70.39, 70.23, 70.19, 68.73, 66.63, 66.46, 44.45, 44.26, 41.48, 39.38, 36.36, 29.94, 29.19, 22.98; LC-MS (ESI): calcd for $C_{64}H_{68}FN_5O_{12}S$: 1150.46420 $[M+H]^+$, found: 1150.21 $[M+H]^+$, R_t = 8.81 min; HR-MS found 1151.46229 $[M+H]^+$.

References

1 Monovich, L. G. *et al.* Discovery of potent, selective, and orally active carboxylic acid based inhibitors of matrix metalloproteinase-13. *J Med Chem* **52**, 3523-3538, doi:10.1021/jm801394m (2009).

2 Huang, X. & Aulabaugh, A. Application of fluorescence polarization in HTS assays. *Methods Mol Biol* **565**, 127-143, doi:10.1007/978-1-60327-258-2_6 (2009).

3 Chidley, C., Haruki, H., Pedersen, M. G., Muller, E. & Johnsson, K. A yeast-based screen reveals that sulfasalazine inhibits tetrahydrobiopterin biosynthesis. *Nature Chemical Biology* **7**, 375-383, doi:Doi 10.1038/Nchembio.557 (2011).

4 Roehrl, M. H., Wang, J. Y. & Wagner, G. A general framework for development and data analysis of competitive high-throughput screens for small-molecule inhibitors of protein-protein interactions by fluorescence polarization. *Biochemistry* **43**, 16056-16066, doi:10.1021/bi048233g (2004).

5 Alexandrov, K., Scheidig, A. J. & Goody, R. S. Fluorescence methods for monitoring interactions of Rab proteins with nucleotides, Rab escort protein, and geranylgeranyltransferase. *Methods Enzymol* **329**, 14-31 (2001).

6 Adveef, A. in *Pharmacokinetic Optimization in Drug Research* 305-326 (VCH, 2001).

7 Kansy, M., Senner, F. & Gubernator, K. Physicochemical high throughput screening: parallel artificial membrane permeation assay in the description of passive absorption processes. *J Med Chem* **41**, 1007-1010, doi:10.1021/jm970530e (1998).

8 Pfaffl, M. W. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res.* **29** (2001).

9 Chen, Y. X. *et al.* Synthesis of the Rheb and K-Ras4B GTPases. *Angew Chem Int Ed Engl* **49**, 6090-6095, doi:10.1002/anie.201001884 (2010).

10 Arosio, D., Manzoni, L., Araldi, E. M. & Scolastico, C. Cyclic RGD functionalized gold nanoparticles for tumor targeting. *Bioconjug Chem* **22**, 664-672, doi:10.1021/bc100448r (2011).