Supplementary Information

A chemical genetic approach for covalent inhibition of analog-sensitive Aurora kinase

André Koch[1#], Haridas B. Rode[2,3#], André Richters[2], Daniel Rauh[2,4*] and Silke Hauf[1*]

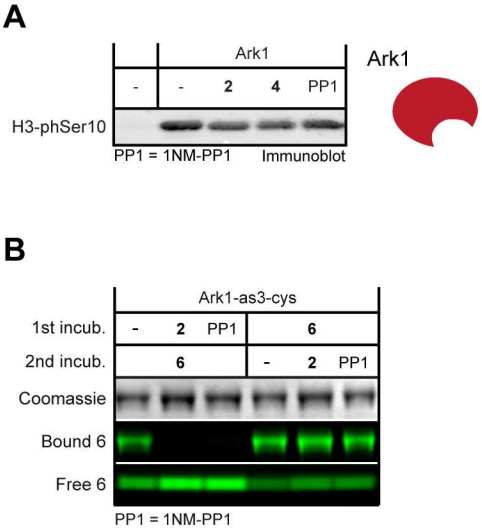
[1]Friedrich Miescher Laboratory of the Max Planck Society, Spemannstrasse 39, D-72076 Tübingen, Germany
[2]Chemical Genomics Centre of the Max Planck Society, Otto-Hahn-Strasse 15, D-44227 Dortmund, Germany
[3]Council of Scientific and Industrial Research (CSIR), Headquarters, 2, Rafi Marg, New Delhi-110001, India
[4]Fakultät Chemie - Chemische Biologie, Technische Universität Dortmund, Otto-Hahn-Strasse 6, D-44227 Dortmund, Germany

[#] both authors contributed equally to this work

*Correspondence: Silke Hauf email: silke.hauf@tuebingen.mpg.de
Phone: +49 7071 - 601 810 Fax: +49 7071 – 601 801

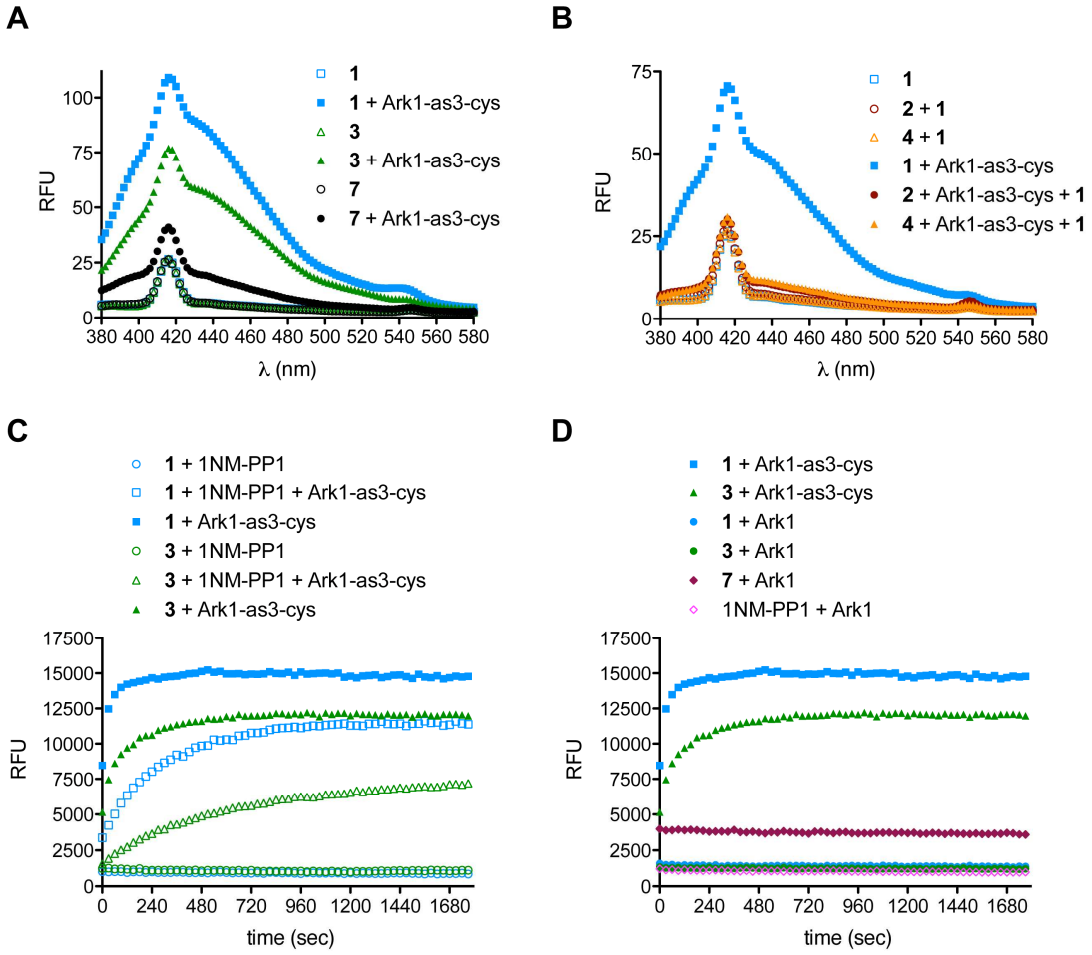
Daniel Rauh email: daniel.rauh@tu-dortmund.de
Phone: +49 231 - 755 7056 Fax: +49 231 - 755 7082

Supplementary Information



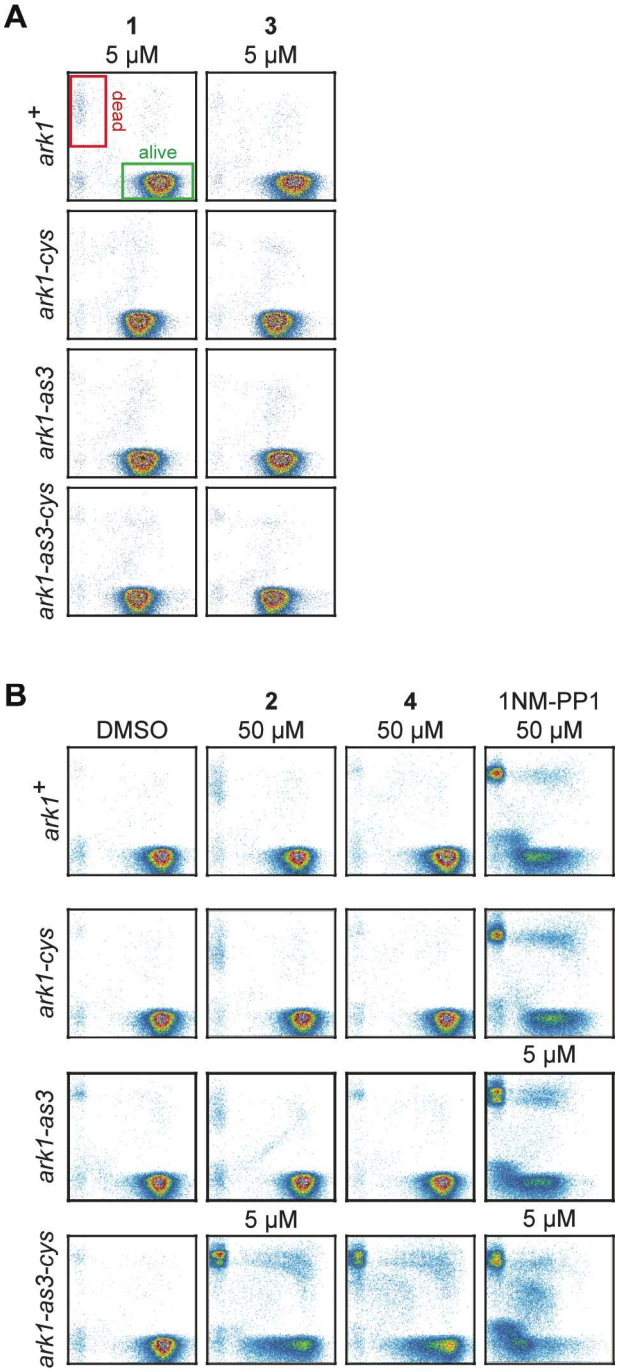
Supplementary Figure 1 Additional characterization of compounds **2**, **4**, and **6**.

(A) Compounds **2** and **4** do not inhibit the kinase activity of wild type Ark1 in a biochemical assay. Kinase assays were performed with recombinant Ark1 that was incubated with 5 µM of the indicated compounds prior to the addition of the substrate (histone H3). The reaction mixtures were separated by SDS-PAGE and immunoblotted with a phospho-specific antibody against Ser10 in histone H3 (H3-phSer10). **(B)** Compound **2** competes for binding of **6** when added before **6**, but not when added after **6**. Recombinantly produced Ark1-as3-cys was pre-incubated with **2**, **6**, or 1NM-PP1 for 5 minutes (1st incub.) followed by incubation with **2**, **6**, or 1NM-PP1 for 5 minutes as indicated (2nd incub.). Reaction mixtures were separated by SDS-PAGE. Ark1 was detected by Coomassie staining. The amount of fluorescent compound **6** co-migrating with Ark1 ('Bound') or present in the running front of the SDS-gel ('Free') respectively are shown. Unlike with **5**, 1NM-PP1 is able to compete for binding of **6**, but only when added before, not when added after **6**. This likely reflects the requirement of **6** to access both selectivity filters for efficient binding.



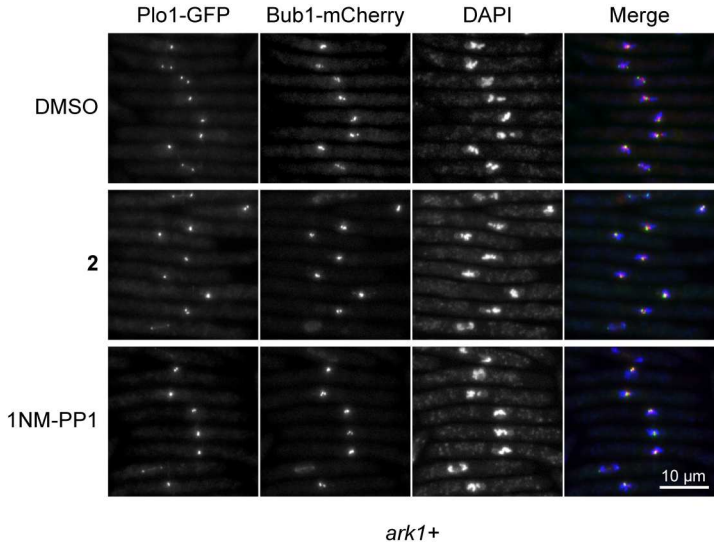
Supplementary Figure 2 Binding of compound **1–4** to Ark1-as3-cys analyzed by fluorescence emission. **(A)** Emission spectra of the quinazoline scaffold of **1** and **3** were measured at 380-580 nm. Emission intensity was significantly increased only when the irreversible inhibitor formed a covalent bond with the Cys anchor point in the ATP-binding site. For **7**, the reversible counterpart of **1** (Supplementary Figure 5), the slight increase in the emission spectra was not due to covalent bond formation since this amplification was also observed when treating Ark1 WT with **7** (data not shown; and see (D)). RFU, random fluorescent units. **(B)** The covalent bond formation of **2** and **4** could not be monitored by fluorescence emission, since these compounds do not change their fluorescent properties upon binding to the protein. To investigate whether these compounds bind to Ark1-as3-cys as proposed, we pre-incubated the kinase with **2** or **4** for 30 min and subsequently added **1**. After another 60 min the change in fluorescence emission was monitored, revealing that **1** alone led to increased fluorescence intensity whereas in combination with **2** or **4** no significant increase was observed providing clear evidence for covalent binding of **2** and **4** to Ark1-as3-cys.

(C) Monitoring fluorescence emission at 420 nm over time allowed for the real-time kinetic analysis of the irreversible inhibition of the Ark1-as3-cys by **1** and **3**. Since the covalent binding event was too fast, competition experiments were performed with 1NM-PP1 to slow down the initial reaction rate. **(D)** Non-specific Cys labeling of Ark1 by **1** and **3** was assessed by monitoring covalent bond formation in real-time over a period of 30 min. Covalent bond formation of **1** and **3** with Cys sulfhydryl of Ark1-as3-cys was detected by an increase in fluorescence emission at 420 nm. Addition of **1**, **3**, **7** or 1NM-PP1 to Ark1 did not result in a time-dependent fluorescence change, suggesting that these do not react non-specifically with solvent-exposed Cys residues.



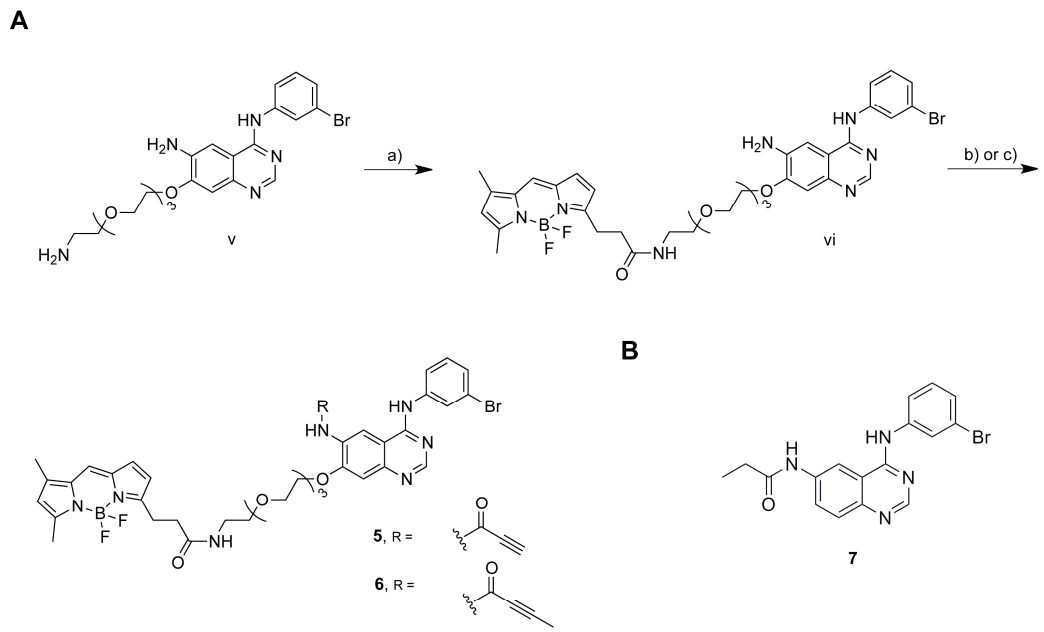
Supplementary Figure 3 Effect of different concentrations of compounds **1** – **4** on cellular viability.

(A, B) Cells in logarithmic growth phase expressing *ark1+*, *ark1-cys*, *ark1-as3* or *ark1-as3-cys* were grown for 4 hours in presence of 5 µM or 50 µM of the indicated compounds, stained with 'Yeast viability kit' (Partec) and 50,000 cells of each treatment were measured in a flow cytometer. Viable cells cluster in the lower right (green box), and dead cells in the upper left (red box) of the scatter plot.



Supplementary Figure 4 Representative pictures of *ark1+* cells from the experiment shown in Figure 4C.

Shown are the Plo1-GFP signal (green in the merged picture), the Bub1-mCherry signal (red in the merged picture) and DNA stained with DAPI (blue in the merged picture).

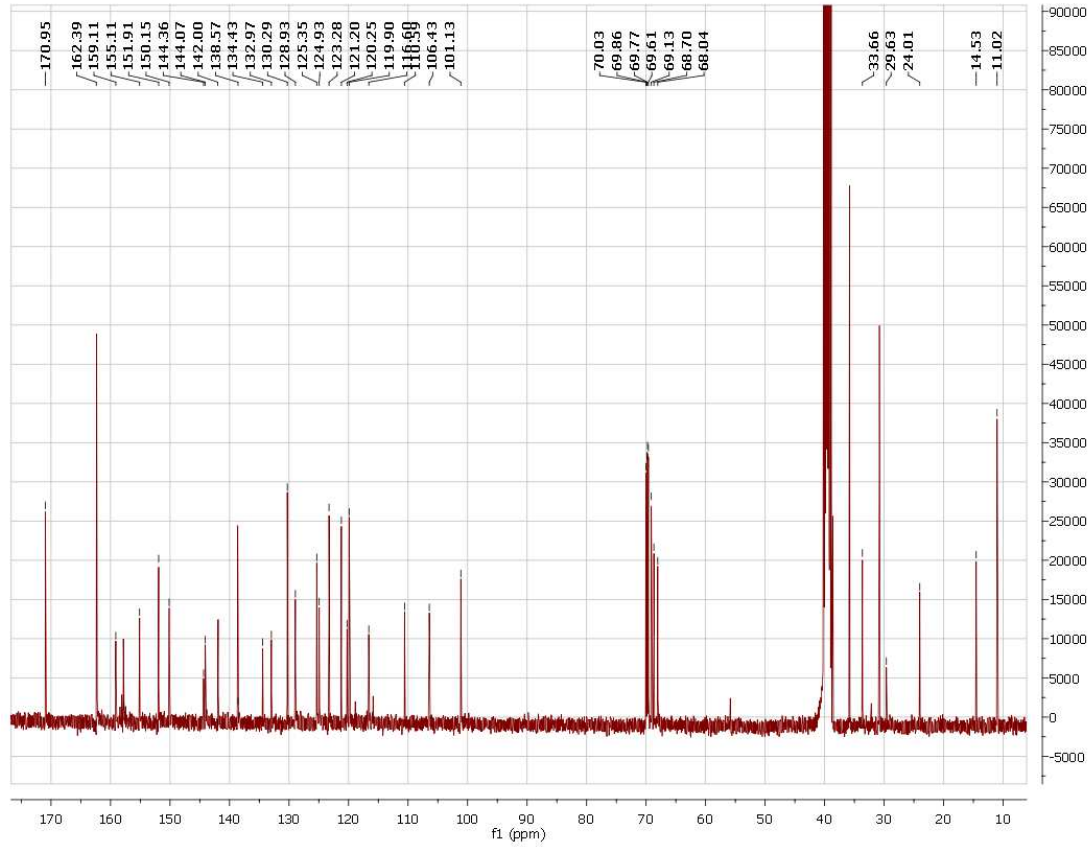


Reagents and conditions: a) 4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-propionic acid, succinimidyl ester, DIPEA, DMF, rt; b) Propiolic acid, EDCI.HCl, 0 °C, DMF, rt; c) 2-Butynoic acid, EDCI.HCl, 0 °C, DMF, rt.

Supplementary Figure 5 Synthesis of fluorescent probes (**5**, **6**), shown in (A), and structure of compound **7**, shown in (B).

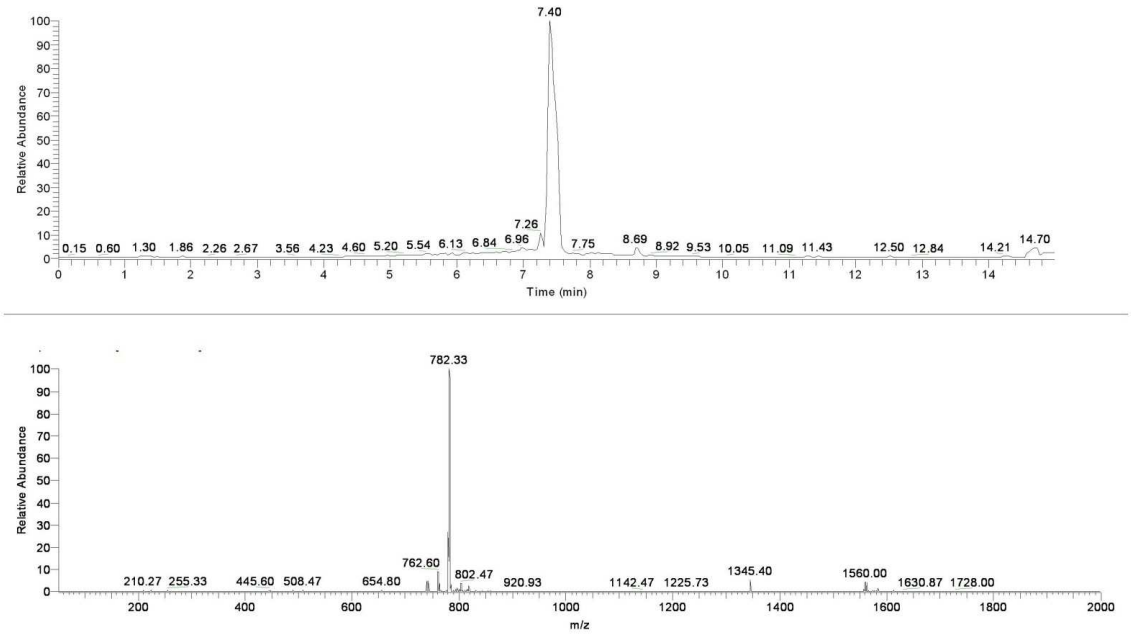
A

B



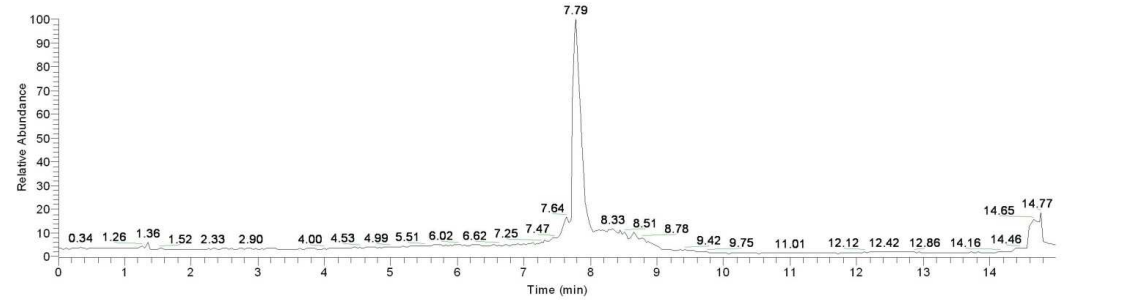
Supplementary Figure 6 (A) ^{1}H-NMR and (B) ^{13}C-NMR data of **vi**.

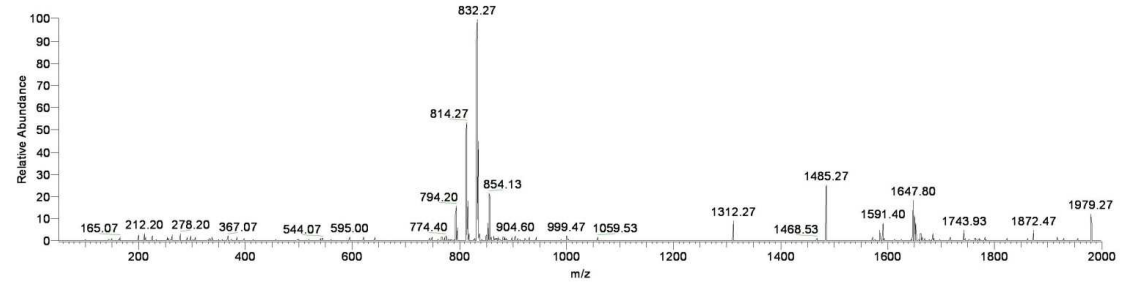
A


Relative Abundance
Time (min)
7.40
7.26
6.96
0.15 0.60 1.30 1.86 2.26 2.67 3.56 4.23 4.60 5.20 5.54 6.13 6.84 7.75 8.69 8.92 9.53 10.05 11.09 11.43 12.50 12.84 14.21 14.70

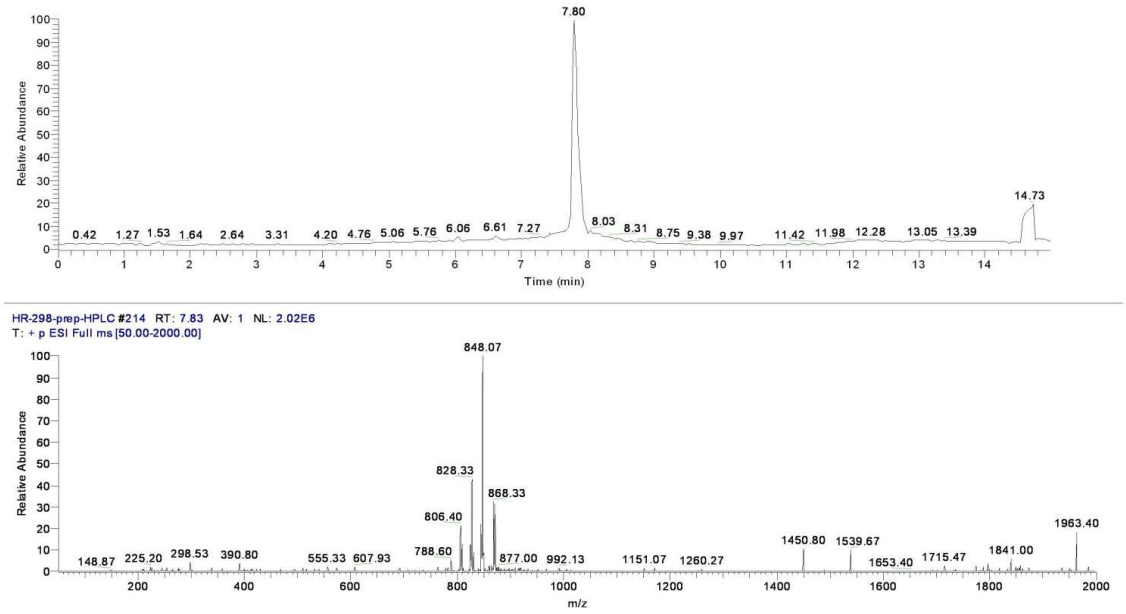
Relative Abundance
m/z
782.33
762.60
802.47
210.27 255.33 445.60 508.47 654.80 920.93 1142.47 1225.73 1345.40 1560.00 1630.87 1728.00

B


Relative Abundance
Time (min)
7.79
7.64
7.47
14.65
14.77
0.34 1.26 1.36 1.52 2.33 2.90 4.00 4.53 4.99 5.51 6.02 6.62 7.25 8.33 8.51 8.78 9.42 9.75 11.01 12.12 12.42 12.86 14.16 14.46


Relative Abundance
m/z
832.27
814.27
854.13
794.20
1485.27
1647.80
1312.27
1591.40
1979.27
165.07 212.20 278.20 367.07 544.07 595.00 774.40 904.60 999.47 1059.53 1468.53 1743.93 1872.47

C



Supplementary Figure 7 LCMS data of **vi** (A), **5** (B) and **6** (C).

Supplementary Table 1 IC_{50} values of investigated compounds.

	IC_{50} (μM)			
	Ark1	**Ark1 + Pic1**	**Ark1-as3-cys**	**Ark1-as3-cys + Pic1**
1	≥ 20.0[a]	≥ 20.0[a]	0.004 ± 0.003	0.002 ± 0.001
2	> 20.0	> 20.0	0.010 ± 0.008	0.001 ± 0.001
3	15.3 ± 1.15	> 20.0	0.012 ± 0.008	0.009 ± 0.007
4	nb[b]	nb[b]	0.004 ± 0.003	0.006 ± 0.002
7	> 20.0	nb[b]	0.272 ± 0.171	0.349 ± 0.147
1NM-PP1	nb[b]	nb[b]	0.006 ± 0.004	0.001 ± 0.001

[a] precipitation of compound at concentrations > 20 µM

[b] nb = non binding: no inhibitory effect at 50 µM

Supplementary Table 2 ATP- and substrate-K_m of Ark1 and Ark1-as3-cys.

	ATP-K_m (μM)	**substrate-K_m (μM)**
Ark1	9.43 ± 0.58	0.103 ± 0.014
Ark1 + Pic1	28.2 ± 4.58	0.054 ± 0.018
Ark1-as3-cys	12.8 ± 1.89	0.207 ± 0.014
Ark1-as3-cys + Pic1	13.7 ± 1.37	0.147 ± 0.018

Supplementary Methods

Measurement of fluorescence emission spectra. Emission spectra were measured on a JASCO FP-6500 fluorescence instrument using 4.5 mL cuvettes (4 clear sides; Carl Roth) containing a suspension of compound (0.15 µM) in the presence and absence of kinase (0.1 µM) in buffer containing 50 mM Tris, pH 7.5, 150 mM NaCl, 0.005% Triton X-100. Inhibitors were pre-incubated with kinase for 60 min to ensure equilibrium was reached.

Real-time detection of covalent bond formation in microtiter plates. The assay was carried out in black 384-well small volume plates and fluorescence emission was measured over time using a TECAN Infinite M1000 microtiter plate reader. Sample wells were excited at 368 nm (slits = 5 nm) and the changing emission intensity was monitored at 420 nm (slits = 20 nm). The reaction was monitored immediately after combining equal volumes (7 µL) of Ark1-as3-cys (1 µM) and inhibitor (1 µM) solution in each well. Competition experiments were carried out by incubating the respective kinase with 1 µM 1NM-PP1 for 15 min prior to adding the inhibitor of interest. In all cases, kinase and inhibitor solutions were prepared using the same buffer described above for measuring fluorescence emission spectra in cuvettes.