Application of Native ESI-MS to Characterize Interactions between Compounds Derived from Fragment-Based Discovery Campaigns and Two Pharmaceutically Relevant Proteins

Originally published in:
SLAS DISCOVERY 23(9), https://doi.org/10.1177/2472555218775921

Supporting Information

Application of native ESI-MS to characterize interactions between compounds derived from fragment based discovery campaigns and two pharmaceutically relevant proteins

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Expression and Purification of CDK2

The kinase domain for human CDK2 with an N-terminal GST-tag, was transformed into E. Coli Rosetta-Gami 2(DE3) cells (Novagen). Cultures were grown for 4–6 h at 37 °C until they reached an optical density at 600 nm (OD600) of 1. At this point the temperature was decreased to 18 °C and 0.1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) was added. The cultures were allowed to grow for an additional 20–24 h at 18 °C and were harvested by centrifugation (15 min at 6000 g). Harvested cells were resuspended to a volume of 200 ml in a buffer with 50 mM HEPES, 150 mM NaCl, 2 mM dithiothreitol (DTT), pH 7.4 containing 0.5 ml protease inhibitors (Roche complete tablet) and 0.5 ml DNAse and sonicated on ice for 10 minutes (5' on / 10' off). Cells were clarified by centrifugation in a JA-14 rotor at 14000 rpm for 1 hour. After sonication and centrifugation, the supernatant was purified by GST-affinity column chromatography (GE LifeSciences). After incubation of peak fractions with GST-PreScission protease (20:1) at 4 °C, the cleaved GST tag was removed by a second GST-affinity column. Fractions containing the protein were loaded onto a Superdex 75 (16/60) column, eluting with a buffer of 25 mM HEPES, 150 mM NaCl, 2 mM DTT, pH 7.4. Purified CDK2 was concentrated to ~10 mg/mL and stored at –80 °C.

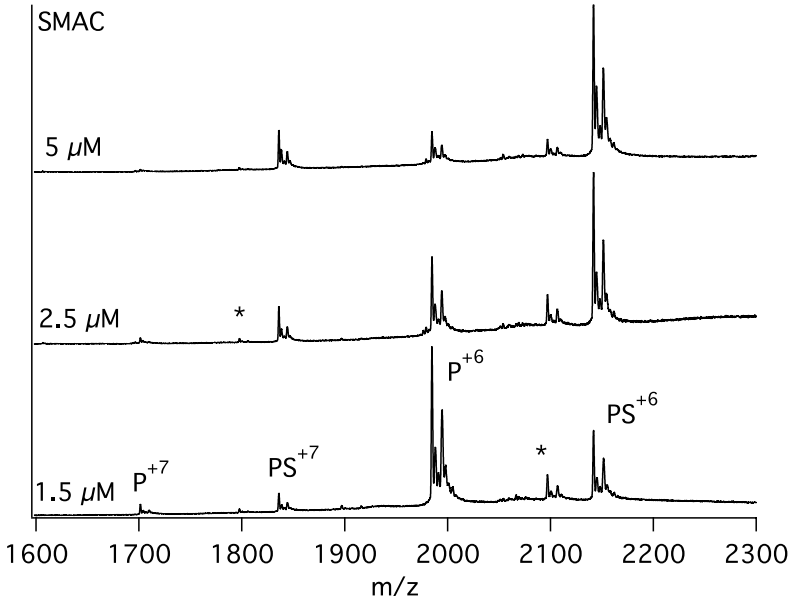


Figure S1: Native ESI-MS spectra of titration of SMAC peptide against 5 μM XIAP. At increased concentration of the SMAC peptide the intensity of the peak of the XIAP-SMAC peptide complex (PS) was increased while that of the protein (P) peak was decreased. The highlighted peak (*) in this and all subsequent XIAP spectra corresponds to the complex of XIAP and a 673 Da peptide that was a component of the bacterial growth medium. However, even at high concentrations of the SMAC peptide this peak was not depleted by competition with the SMAC peptide.

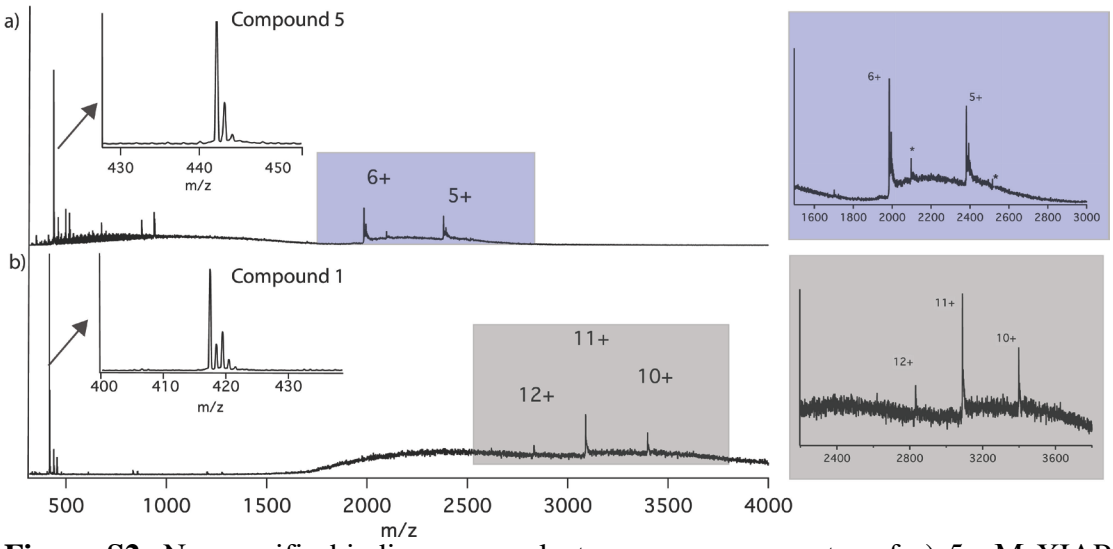


Figure S2: Nonspecific binding - nanoelectrospray mass spectra of a) 5 μM XIAP and 50 μM compound **5** and b) 5 μM CDK2 and 50 μM compound **1**. No ligand binding to the protein was observed in either case. The most abundant peak in both spectra was that corresponding to free compound. The m/z ranges of the protein spectra are highlighted in the zoomed spectra on the right.

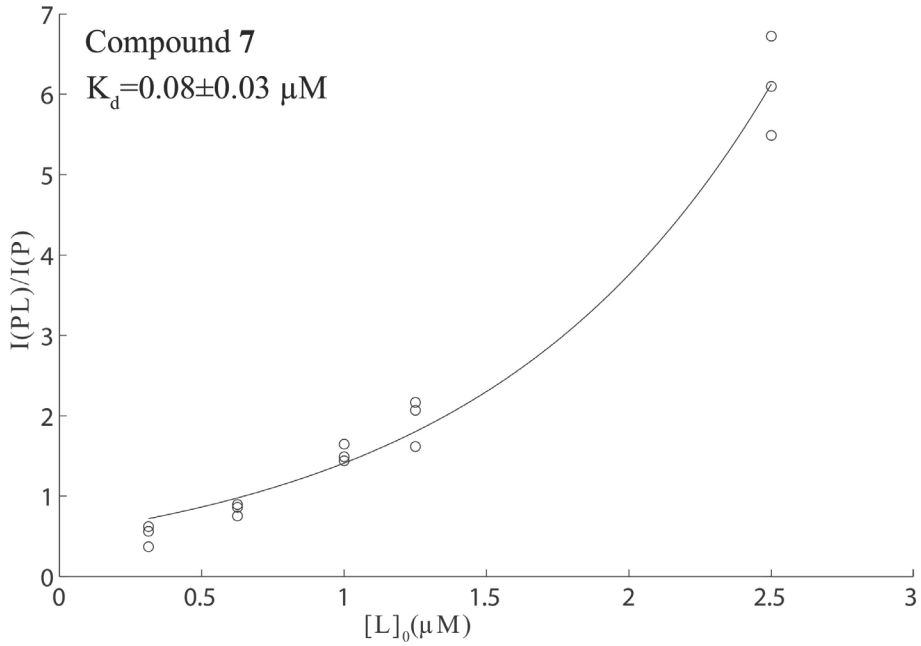


Figure S3: Titration curve from triplicate measurements of the compound 7 to 5 µM CDK2 in 50 mM NH4Ac, 1 % DMSO. The measured relative peak areas (I) of the complex (PL) to the protein (P) are plotted versus the initial concentration of the compound ([L]o).

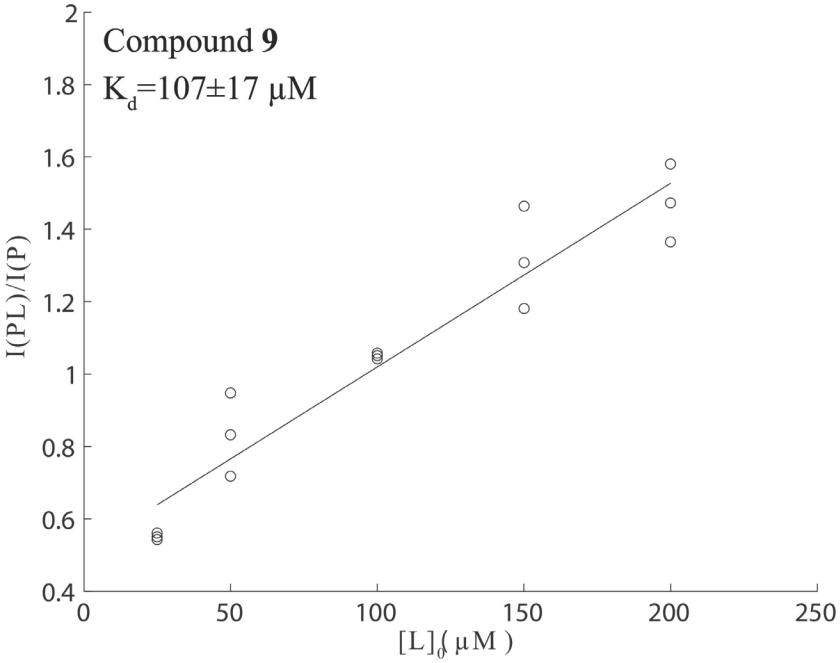


Figure S4: Titration curve from triplicate measurements of compound **9** against 5 µM CDK2 in 50 mM NH_4Ac, 1 % DMSO. The measured relative peak areas (I) of the complex (PL) to the protein (P) are plotted versus the initial concentration of the compound ($[L]_o$). For weak affinities with K_d values > 100 µM the quality of the fit is reduced.

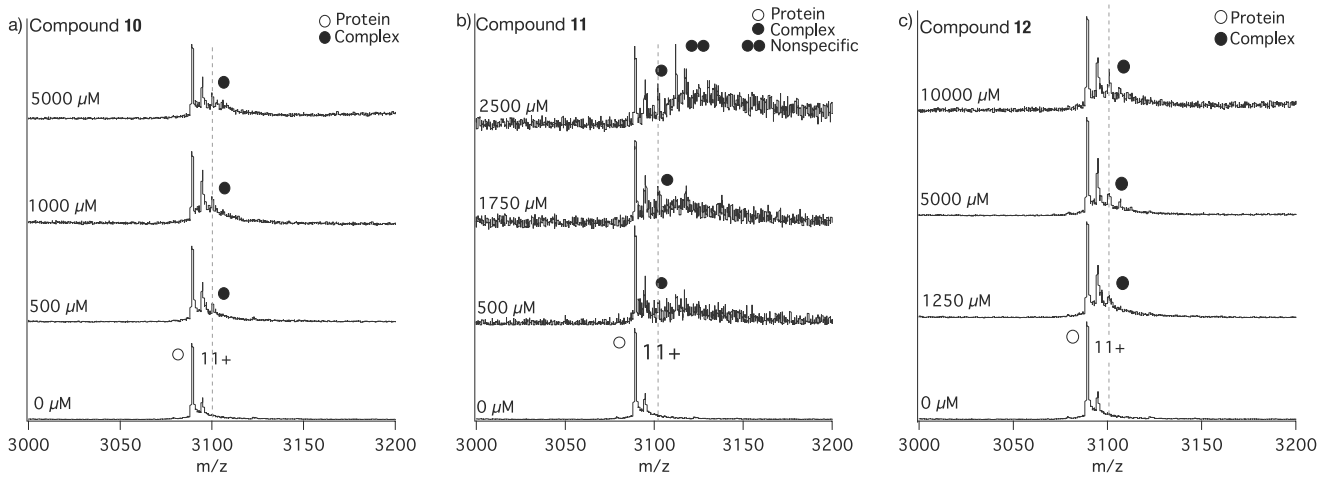


Figure S5: Native ESI-MS spectra for the 11+ charge state of the titration of the three compounds with the lowest affinity, **10-12** against 5 μM CDK2 in 50 mM NH_4Ac, 1% DMSO

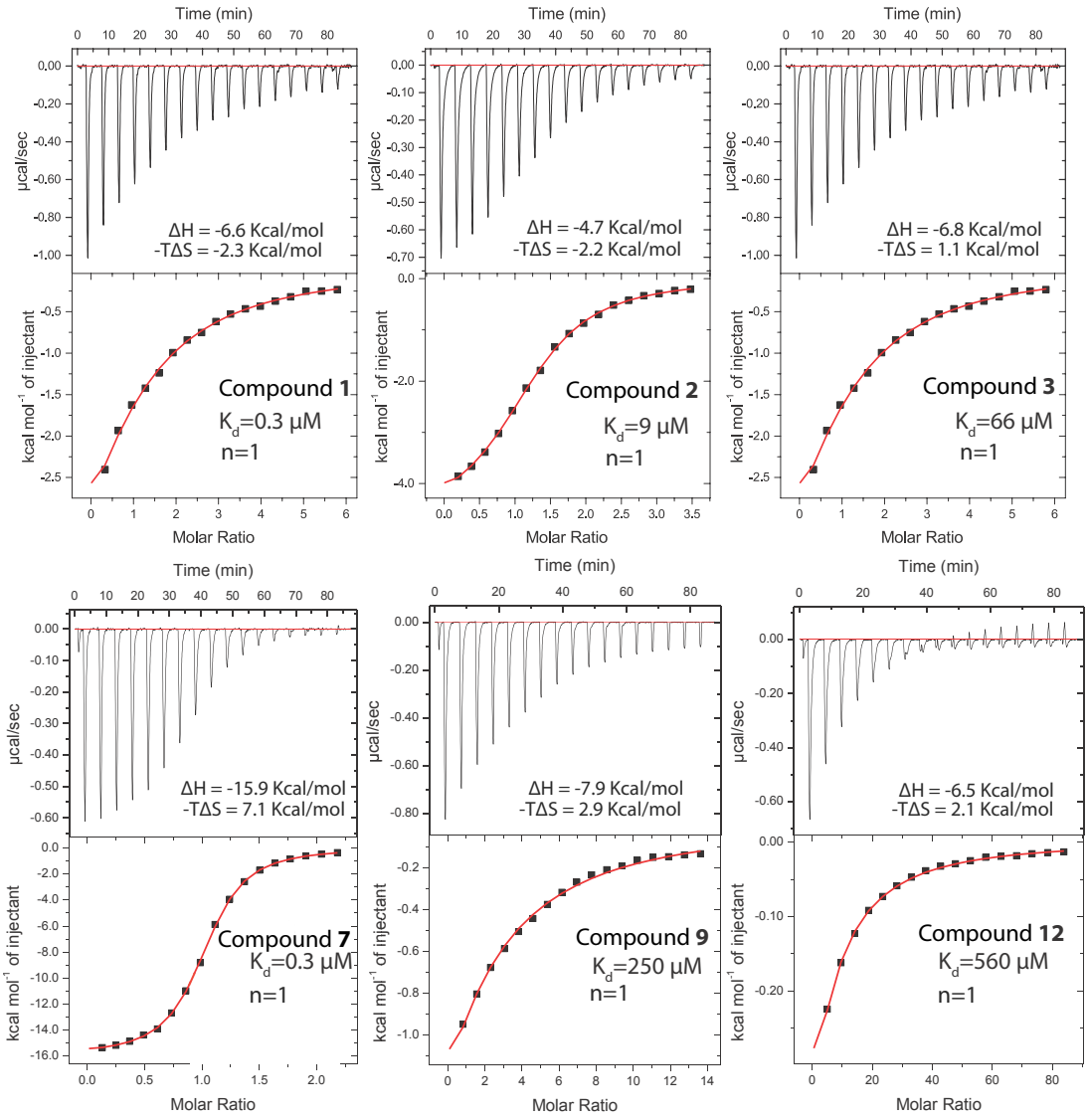


Figure S6: Measurement of thermodynamic parameters including dissociation constant (K_d) and stoichiometry (n) by ITC for interactions between XIAP with compounds **1-3** and CDK2 with compounds **7**, **9**, **12**.

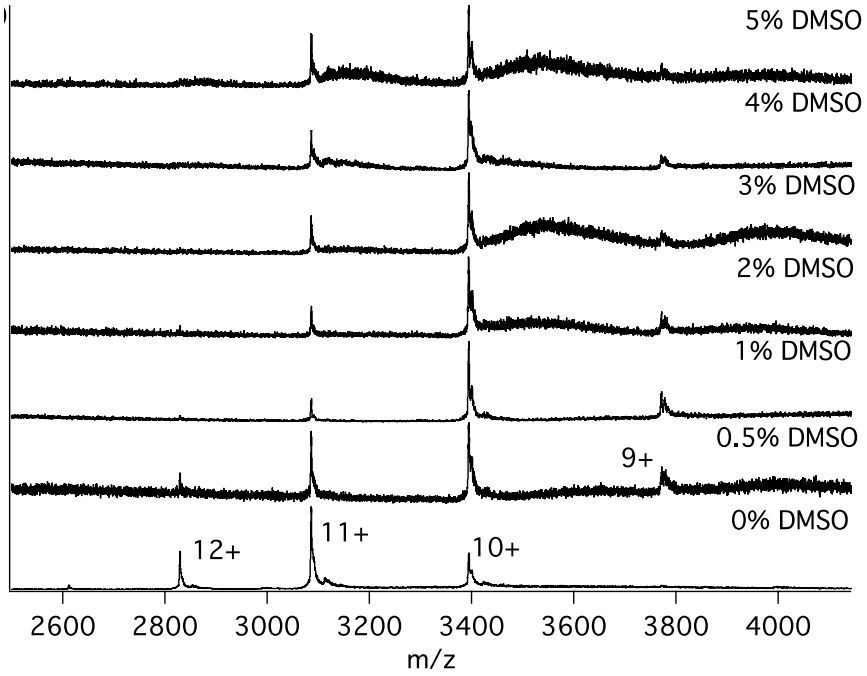


Figure S7: Nanoelectrospray mass spectra of 5 μM CDK2 in 50 mM NH_4Ac containing 0-5 % DMSO.

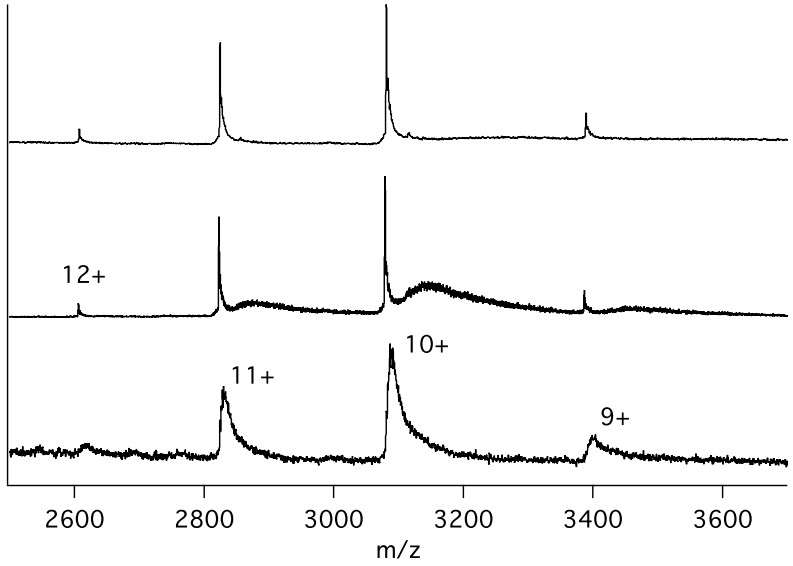


Figure S8: Nanoelectrospray mass spectra of 5 μM CDK2 in 50 mM NH_4Ac after one desalting step (lower spectrum), two desalting steps (middle spectrum) and three desalting steps (upper spectrum). The S/N ratio increased sequentially with successive desalting steps.

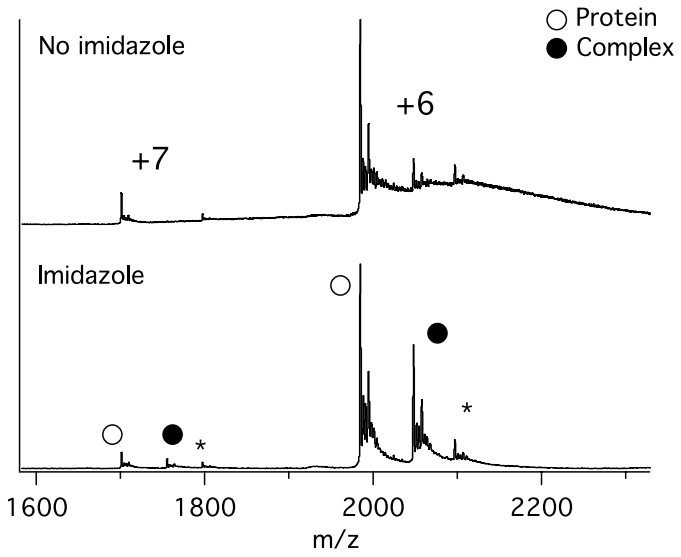


Figure S9: Representative nano ESI-MS spectra of 5 μM XIAP in complex with 50 μM antagonist, +/- 10 mM imidazole in 20 mM NH_4Ac, pH 7.5. The lower spectrum was recorded after addition of imidazole.

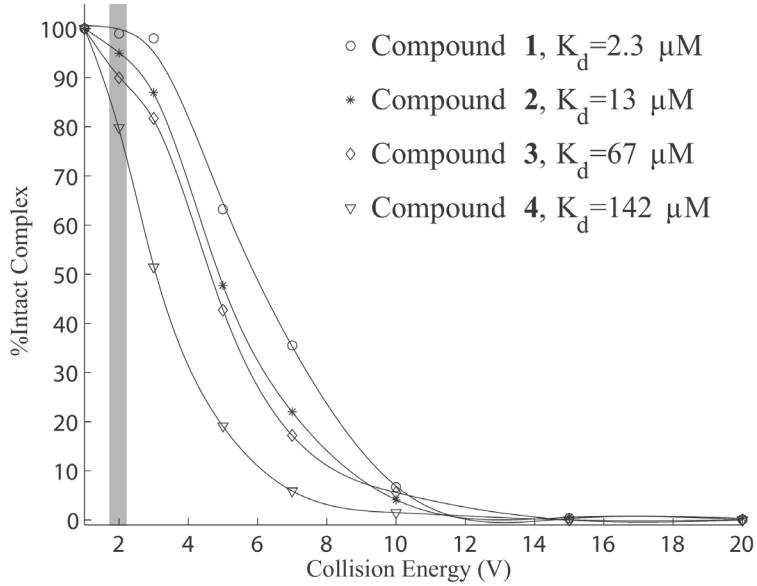
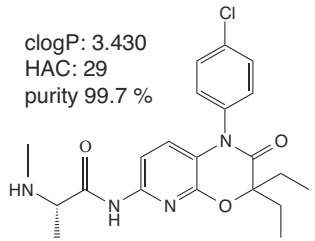
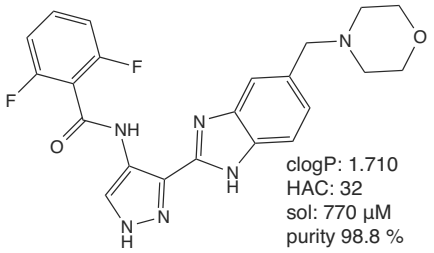
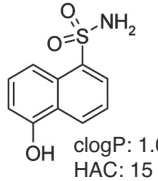
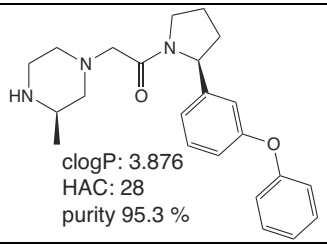
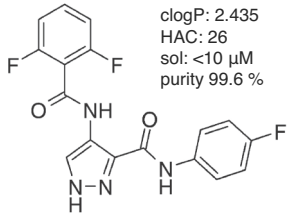
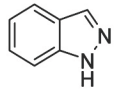
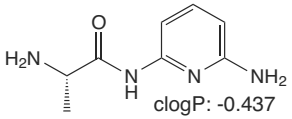
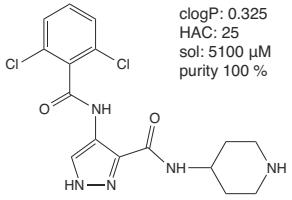
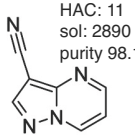
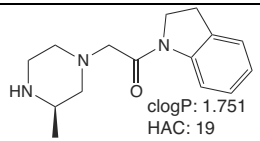
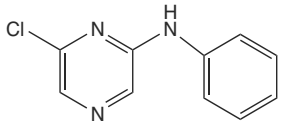
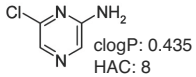


Figure S10: Dissociation curves for the various noncovalent XIAP complexes: relative intensity of the complex plotted as a function of the collision energy to show gas-phase stabilities of the complexes. The optimum collision energy of 2 V is highlighted.

Table S1. Compounds, molecular weight (MW), structures, clogP, the number of heavy atoms (heavy atom count, HAC), solubility (sol) in 50 mM NH_4Ac / 1% DMSO, pH 7.5 where available, and the purity (in %) of the compounds. * Fragment hits; ** Compounds in the hit-to-lead phase; * Compounds in the lead optimization phase.**

Compound	MW (Da)	Structure	Compound	MW (Da)	Structure	Compound	MW(Da)	Structure
1 **	416	 clogP: 3.430 HAC: 29 purity 99.7 %	**5** ***	438	 clogP: 1.710 HAC: 32 sol: 770 µM purity 98.8 %	**9** *	223	 clogP: 1.090 HAC: 15 sol: 770 µM purity 89.7 % [2]
2 **	379	 clogP: 3.876 HAC: 28 purity 95.3 %	**6** ***	360	 clogP: 2.435 HAC: 26 sol: <10 µM purity 99.6 %	**10** *	118	 clogP: 1.625 HAC: 9 sol: 4810 µM purity 98.8 %
3 *	180	 clogP: -0.437 HAC: 13 purity 88.8 % [1]	**7** ***	381	 clogP: 0.325 HAC: 25 sol: 5100 µM purity 100 %	**11** *	144	 clogP: -0.157 HAC: 11 sol: 2890 µM purity 98.1 %
4 **	259	 clogP: 1.751 HAC: 19 purity 98 %	**8** **	205	 clogP: 3.240 HAC: 14 sol: 190 µM purity 99.5 %	**12** *	129	 clogP: 0.435 HAC: 8 sol: 5210 µM purity 100 %

[1] Major impurity: tert-butyloxycarbonyl (BOC) protecting group

[2] Major impurity: dimer of compound 9

Table S2: Measured and calculated masses of the proteins and their noncovalent complexes

Proteins/Mass	Compound theoretical mass (Da)	Measured mass of noncovalent complexes (Da)	Measured ΔM = Mass of complex –Mass of protein (Da)
XIAP Theoretical: 11840 Da Measured: 11904 ± 2 Da[1]	Compound **1:** 416	12324 ± 3	420
	Compound **2:** 379	12288 ± 3	384
	Compound **3:** 180	12089 ± 5	185
	Compound **4:** 259	12164 ± 3	260
CDK2 Theoretical: 33929 Da Measured: 33891 ± 5 Da	Compound **5:** 438	34323 ± 2	432
	Compound **6:** 360	34251 ± 1	360
	Compound **7:** 381	34271 ± 3	380
	Compound **8:** 205	34101 ± 2	210
	Compound **9:** 223	34120 ± 6	229
	Compound **10:** 118	34012 ± 7	121
	Compound **11:** 144	34035 ± 6	140
	Compound **12:** 129	34023 ± 14	132

[1] The difference between the measured and theoretical mass is due to the zinc ion (65 Da).