STRUCTURAL
BIOLOGY

Volume 77 (2021)

Supporting information for article:

Frag4Lead: growing crystallographic fragment hits by catalog using fragment-guided template docking

Alexander Metz, Jan Wollenhaupt, Steffen Glöckner, Niki Messini, Simon Huber, Tatjana Barthel, Ahmed Mehrabed, Hans-Dieter Gerber, Andreas Heine, Gerhard Klebe and Manfred S. Weiss

Table S1. Acquired follow-up candidates, affinities, providers and purities.

Follow-Up Compound	*LE*[a]	K_d[b] [µM]	CAS Number	Salt	Provider	Provider Catalog ID	Purity[c] [%]
FU$_5$-1 · HCl	0.39	6.4	40227-56-3	1xHCl	resynthesized	resynthesized	98.5[d]
FU$_5$-1	[e]	[e]	46886-52-6		Vitas-M	STL231607	90[f]
FU$_5$-2	0.37	970	3468-11-09		AK Scientific	P175	95
FU$_5$-3	0.39	740	14352-51-3		ENAMINE	EN300-43093	90
FU$_5$-4	0.31	400	879916-45-7		Vitas-M	STK594879	90[f]
FU$_5$-5			571161-36-9		Vitas-M	STK583580	90[f]
FU$_{41}$-1			919949-40-9		ENAMINE	Z106906918	90
FU$_{41}$-2			1007745-42-7		UORSY	PB106986608	n/a
FU$_{41}$-3			850020-65-4		ENAMINE	Z46204096	90
FU$_{41}$-4			1105566-03-7		ENAMINE	Z46442873	90[f]
FU$_{41}$-5			702651-79-4		ChemDiv	C094-0062	n/a[f]
FU$_{41}$-6			793678-62-3		ENAMINE	Z44502465	90
FU$_{58}$-1	0.17	450	1377929-97-9		ChemBridge	57162056	90
FU$_{58}$-2			1331991-20-8		ChemBridge	69271435	n/a
FU$_{58}$-3	(0.19)[g]	(1040)[g]	1244921-80-9		ChemBridge	58887845	90
FU$_{58}$-4			1360217-23-7		ChemBridge	22615646	90
FU$_{58}$-5			1332207-28-9		ChemBridge	55009691	n/a
FU$_{58}$-6			1378100-25-4		ChemBridge	17104240	n/a
FU$_{58}$-7			1269257-22-8		ChemBridge	33257899	n/a
FU$_{58}$-8			1005631-30-0		Vitas-M	STL398417	n/a[f]
FU$_{58}$-9			1355873-93-6		ENAMINE	Z1156178129	90
FU$_{66}$-1	0.21	160	1355830-29-3		ENAMINE	Z906038686	90
FU$_{66}$-2			2034580-57-7		Life Chemicals	F6482-6629	90
FU$_{66}$-3			2034475-57-3		Life Chemicals	F6482-6686	90
FU$_{66}$-4			2034307-19-0		Life Chemicals	F6482-6819	90
FU$_{66}$-5			1375192-22-5		ENAMINE	Z908233534	90
FU$_{66}$-6			1375215-10-3		ENAMINE	Z907996506	90
FU$_{290}$-1	0.44	7.2	10465-18-6	2xHCl	ChemBridge	5111431	90[f]
FU$_{290}$-2	0.40	160	1417634-38-8	1xHBr	Vitas-M	STL301596	n/a

[a] Ligand efficiency in kcal mol^{-1} atom^{-1}

[b] K_d value determined by ITC

[c] Purity reported by MolPort if not stated otherwise. “n/a”: no purity information available

[d] Determined by ^{1}H-qNMR

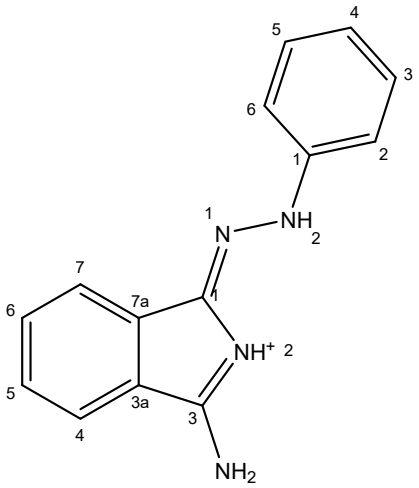
[e] Affinity only determined for resynthesized **FU$_5$-1** · HCl

[f] Identity verified by NMR provided by vendor

[g] Measurement is not reliable as crystallographic finding indicates only binding of impurity

Synthesis of compound FU_5-1

i.e. (Z)-3-amino-1-(2-phenylhydrazineylidene)-1*H*-isoindol-2-ium chloride



(*Z*)-3-amino-1-(2-phenylhydrazineylidene)-1 *H*-isoindol-2-ium
Chemical Formula: $C_{14}H_{13}N_4^+$
Exact Mass: 237.11
Molecular Weight: 237.29
m/z: 237.11 (100.0%), 238.12 (15.1%), 238.11 (1.5%), 239.12 (1.1%)
Elemental Analysis: C, 70.87; H, 5.52; N, 23.61

The target compound (**FU_5-1** · HCl) was prepared by a slightly modified literature procedure (Biitseva et al. 2013, Wolf, W. & Vollmann, H. 1956) as follows:

A stirred mixture of 1,3-diiminoisoindole (0.73 g, 5.0 mmol) and phenylhydrazine (0.54 g, 5.0 mmol) in MeOH (30 mL) was refluxed for 2 h. After cooling to RT, aqueous 1 M HCl (60 mL, 60 mmol) was added and the mixture stirred for 15 min at RT. The resulting yellow-orange precipitate was filtered through a glass filter (G3), thoroughly washed with water, then with a 1:1 mixture of water/MeOH and finally dried *in vacuo* over P_4O_{10} giving rise to 0.92 g (67.5 %) of a yellow-orange solid.

m.p. 228 °C (decomp.; (Biitseva *et al.*, 2013): 172 °C); at ca. 175 °C morphism into needles! ^{1}H NMR (DMSO-d_6, 500.1 MHz): δ = 13.30 (bs, 1H), 11.41 (bs, 1H), 10.61 and 10.36 (2 x bs, merged, 2H), 8.29 (d, $^3J_{H,H}$ = 7.8 Hz, 1H), 7.97 (d, $^3J_{H,H}$ = 7.6 Hz, 1H), 7.82 (t, $^3J_{H,H}$ = 7.8 Hz, 1H), 7.65 (t, $^3J_{H,H}$ = 7.6 Hz, 1H), 7.37-7.27 (m, 4 H), 6.95-6.87 (sm, 1H). ^{13}C NMR (DMSO-d_6, 100.5 MHz): δ = 158.6, 144.0, 134.4, 134.2, 131.2, 129.2, 129.1, 125.7, 124.2, 120.7, 120.5, 112.7. MS (ESI+) *m/z* (%): 237 (93) $[M]^+$. HRMS (ESI+): *m/z* calculated for $C_{14}H_{13}N_4^+$ 237.1135 $[M]^+$; found 237.1139. Elemental analysis calculated (%) for $C_{14}H_{13}N_4Cl$ (272.74): C 61.65, H 4.80, N 20.54, Cl, 13.00; found: C, 61.13; H, 5.04; N, 20.27; Cl, 12.86

Substance assay, determined by ^{1}H qNMR (DMSO-d_6, 500.1 MHz, reference standard maleic acid TraceCert®): 98.5 %

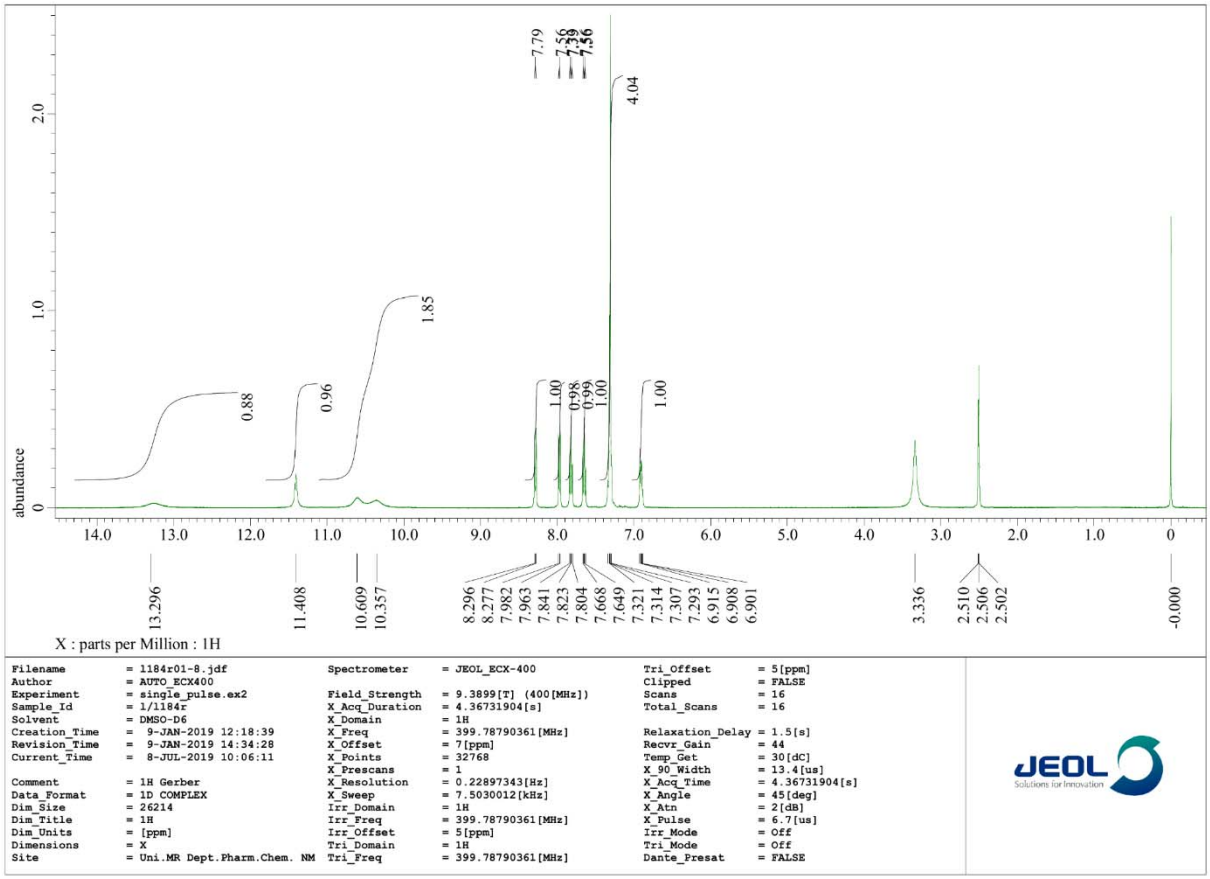


Figure S1. ^{1}H NMR spectrum of **FU$_5$-1** · HCl.

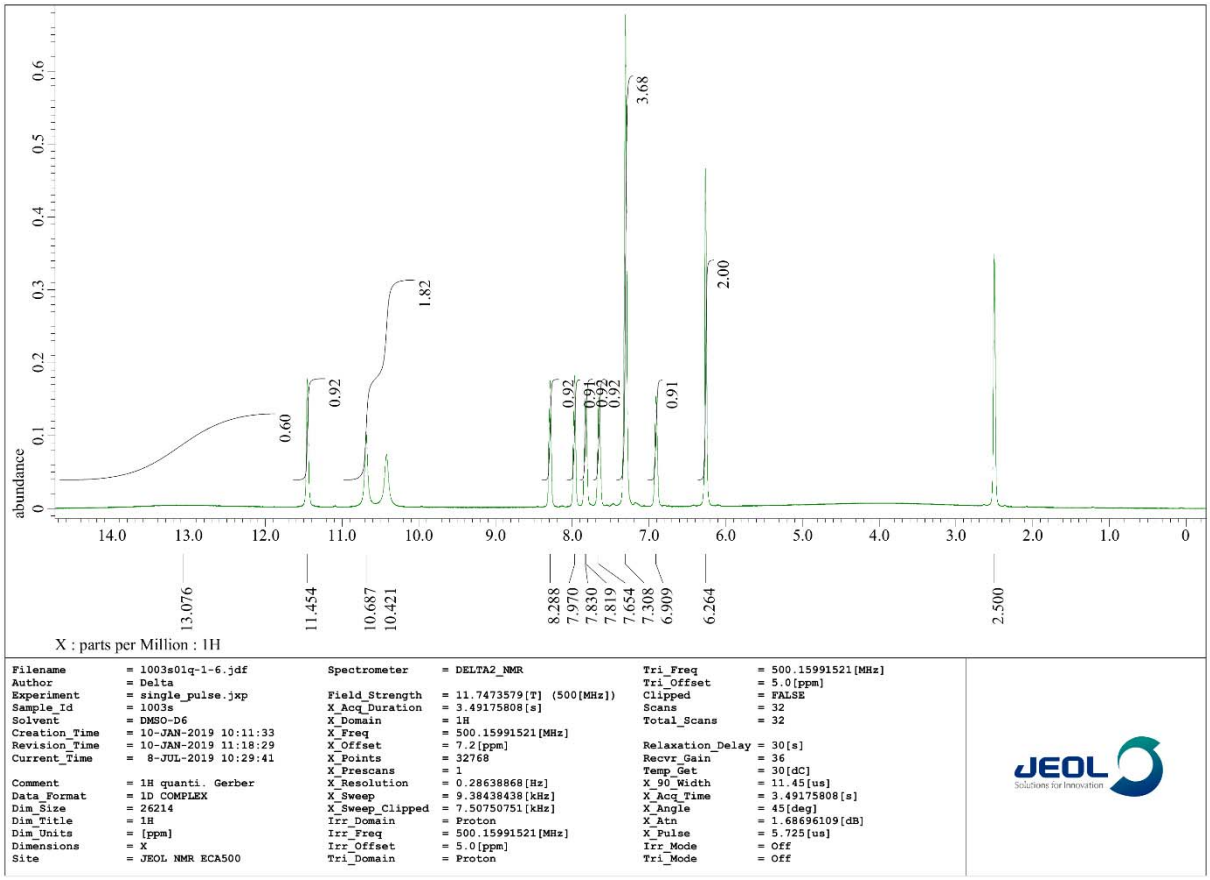


Figure S2. ^{1}H qNMR spectrum of **FU$_5$-1** · HCl.

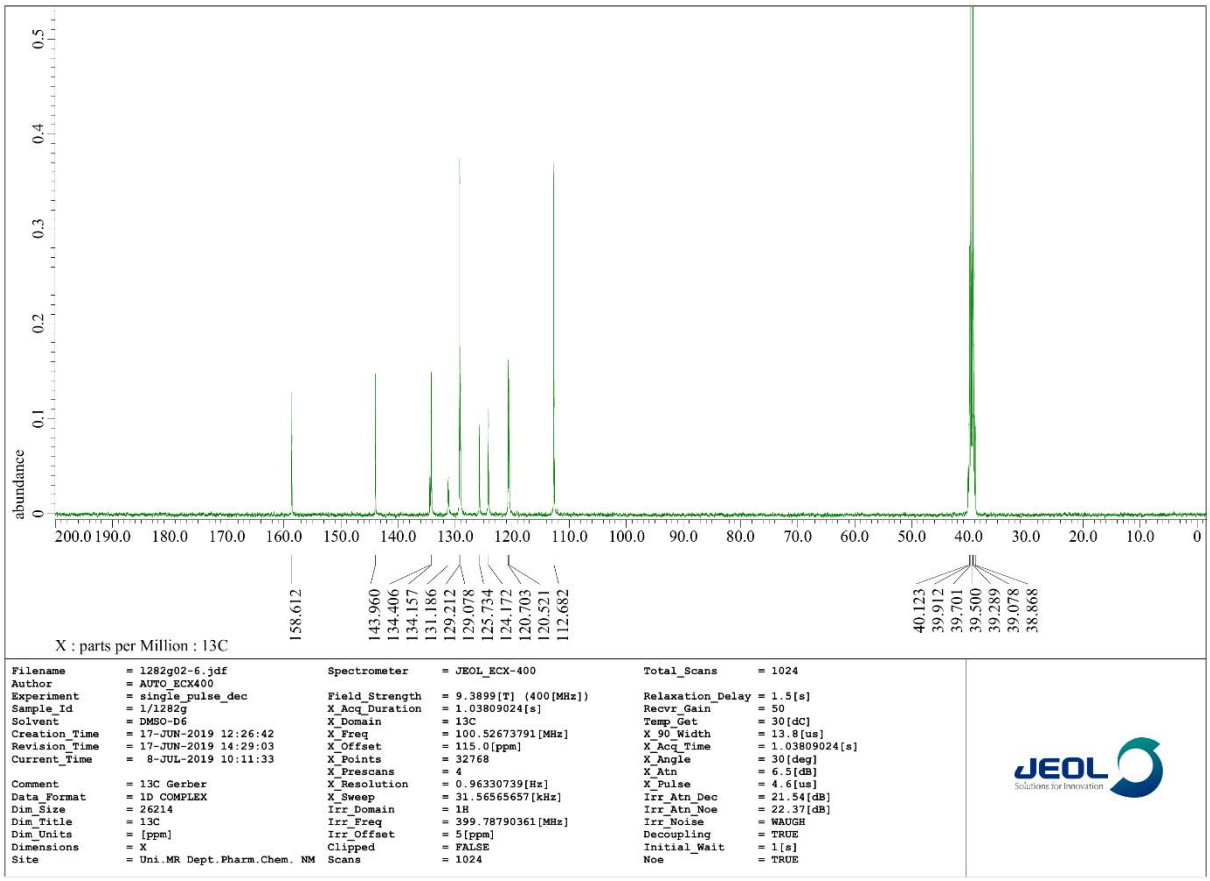


Figure S3. ^{13}C NMR spectrum of **FU$_5$-1** · HCl.

Table S2. Details of ITC measurements.

Follow-Up Compound	Titration Type	Protein Concentration in Cell	Follow-Up Ligand Concentration in Cell	Ligand Concentration in Syringe	Final Stoichiometry (Ligand:Protein)
FU$_5$-1 · HCl	direct	25 µM[a]	–	500 µM[a]	4
FU$_5$-2	displacement	50 µM	2000 µM	500 µM	2
FU$_5$-3	displacement	50 µM	2000 µM	500 µM	2
FU$_5$-4	direct	50 µM	–	2000 µM	8
FU$_{58}$-1	displacement	50 µM	2000 µM	500 µM	2
FU$_{58}$-2[b]	–	–	–	–	–
FU$_{58}$-3	displacement	50 µM	2000 µM	500 µM	2
FU$_{66}$-1	displacement	50 µM	2000 µM	500 µM	2
FU$_{290}$-1	direct	50 µM	–	1000 µM	4
FU$_{290}$-2	displacement	50 µM	2000 µM	500 µM	2

[a] 0.1% (v/v) Tween 20 in syringe and cell.

[b] Not enough substance available for ITC.

Table S3. Fitting statistics for ITC titration curves

Compound	Global reduced chi-square[a]	Weighted local rmsd[a]	Stochiometry[b] (B:A)	n[c]	K_d [µM] (SD)[d]
FU$_5$-1[e]	0.026	28.6	0.875	25	6.4 (± 0.06)
FU$_5$-1[e]	0.024	38.7	0.915	25	6.4 (± 0.06)
FU$_5$-1[e]	0.003	37.7	0.858	25	6.4 (± 0.06)
FU$_5$-2	0.228	135.3	0.991	25	970 (± 79)
FU$_5$-2	0.357	268.2	0.972	25	970 (± 79)
FU$_5$-2	0.284	238.2	1.018	25	970 (± 79)
FU$_5$-3	0.107	240.5	1.052	13	740
FU$_5$-4	0.014	18.6	1.000[g]	38	400
FU$_{58}$-1	0.134	197.5	1.081	24	450
FU$_{58}$-3	0.498	396.7	1.068	24	1040
FU$_{66}$-1	0.052	226.0	1.142	24	160
FU$_{290}$-1	0.075	186.9	0.816	74	7.2
FU$_{290}$-2	0.065	41.4	0.871	25	160
Reference (SAP114)	0.346	193.2	1.001	24	680

Isotherm fitting statistics of ITC data, analyzed via SEDPHAT 10.58d (Houtman *et al.*, 2007).
[a] Goodness of fit parameters (global reduced chi-square and weighted local rmsd) determined with SEDPHAT 10.58d are comparable to those listed for the established reference compound SAP114.
[b] Stochiometry of the ligand protein A and the ligand B at the inflection point of the sigmoidal fitting curve
[c] Number of used data points corresponding to injections of the ITC experiment
[d] K_d values. Standard deviation (SD) is only listed for **FU$_5$-1** and **FU$_5$-2**, which were measured as triplicates.
[e] For **FU$_5$-1**, global reduced chi-square and weighted local rmsd are listed for comparison only and the listed K_d value was determined by global fit with the AFFINImeter suite (version 2.1710; Muñoz & Piñeiro, 2018).
[f] For **FU$_5$-4** the stoichiometry was fixed to a value of 1 to achieve a satisfactory fit

ΔH and $T\Delta S$ values are not reported for this experiment following the reasoning of Krimmer and Klebe (2015).

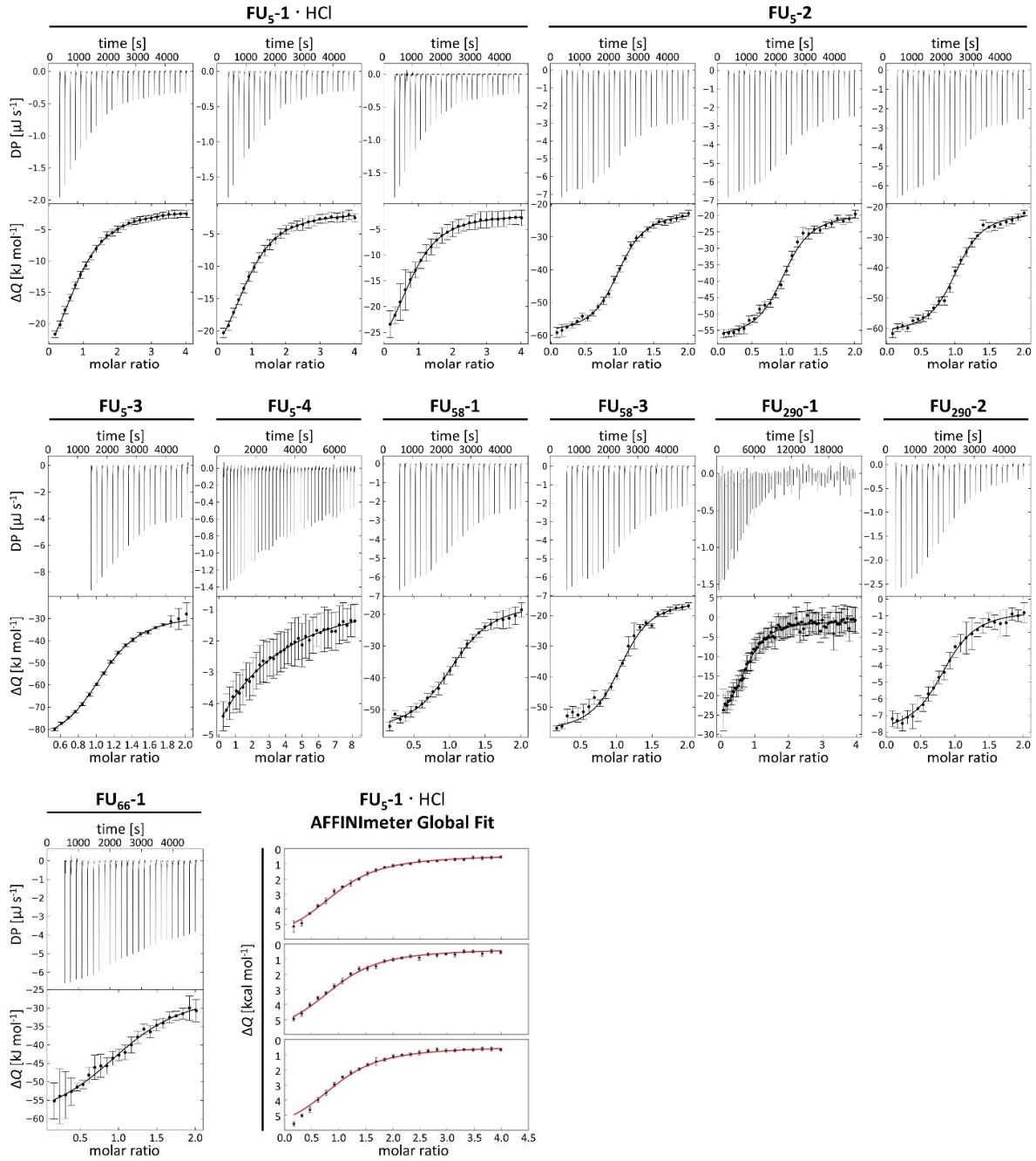


Figure S4. ITC thermograms for crystallographically identified follow-up ligands. In all cases, the heat signals DP (in µJ s^{-1}) as a response by the release of the ligand into the protein solution is shown over the course of the experiment along with the integrated heat signals of the injections ΔQ (in kJ mol^{-1}). **FU$_5$-1** · HCl and **FU$_5$-2** were measured in triplicate, all other ligands as single measurements (n = 1), in due regard of the limited resilience of the results of the latter. The noisy baseline of the ITC experiments for **FU$_{290}$-1** was allegedly due to its low purity (> 90% according to provider) and could not be further improved.

Mass Spectrometry Analysis of FU_{58}-3

Clear difference electron density and steric constraints in the crystal structure obtained by soaking **FU_{58}-3** showed that **FU_{58}-3b** had bound instead. The presence of **FU_{58}-3b** in the commercially obtained sample of **FU_{58}-3** (> 90% according to provider (ChemBridge, USA)) was demonstrated by mass spectrometry (MS) experiments (Figure S6) using a Q-Trap 2000 (Applied Biosystems, USA).

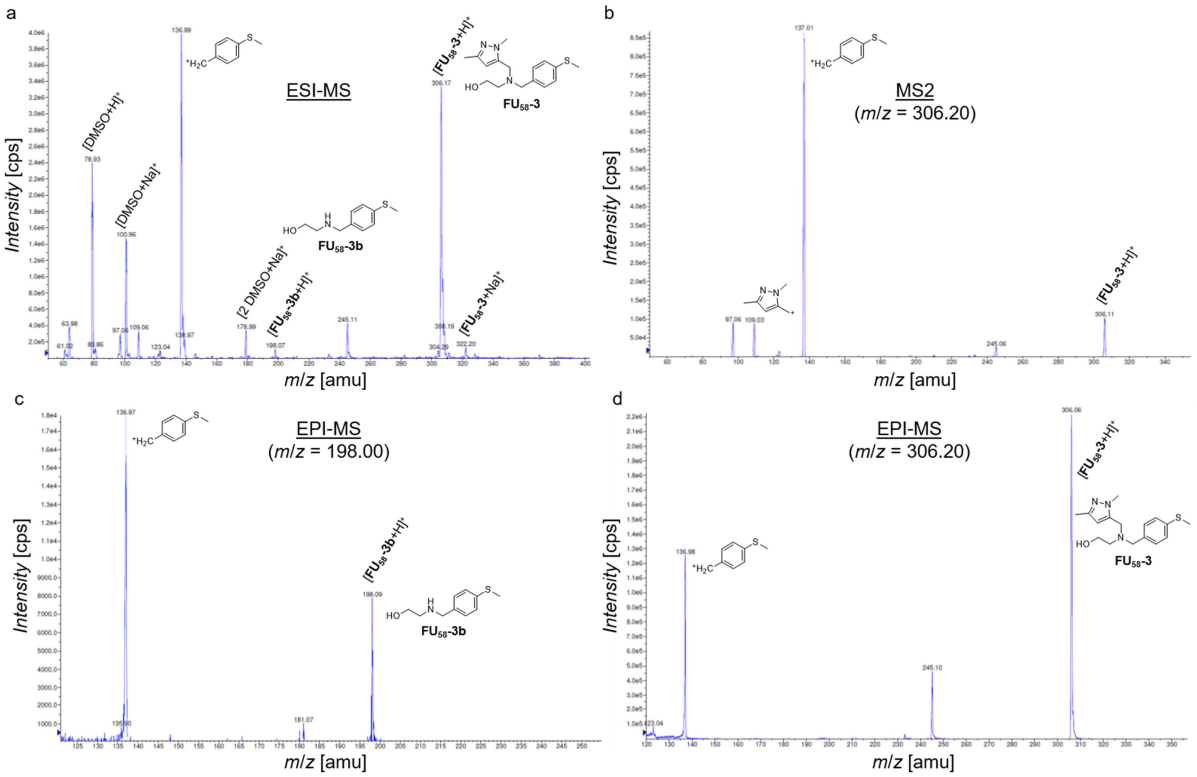


Figure S5. Mass spectrometry results of commercially obtained sample of **FU_{58}-3**. a) ESI-MS. b) MS2. c) EPI-MS targeting *m/z* = 198.00. c) EPI-MS targeting *m/z* = 306.20.

In detail, **FU_{58}-3** was dissolved in methanol from 1 M DMSO stock and characterized by electrospray ionization (ESI) MS (Figure S6a). This demonstrated the presence of **FU_{58}-3** (calcd. for $C_{16}H_{24}N_3OS$ $[M+H]^+$ 306.16, found m/z = 306.17) and its substructure **FU_{58}-3b** (2-((4-(methylthio)benzyl)amino)ethan-1-ol) (calcd. for $C_{10}H_{16}NOS$ $[M+H]^+$ 198.09, found m/z = 198.07). In addition, MS2 targeting **FU_{58}-3** (m/z = 306.20; Figure S6b) demonstrated its presence (m/z = 306.11) as well as the presence of its fragmentation products (4-(methylthio)phenyl)methylium (calcd. for C_8H_9S $[M]^+$ 137.04, found m/z = 137.01) and (1,3-dimethyl-1H-pyrazol-5-yl)methylium (calcd. for $C_6H_9N_2$ $[M]^+$ 109.08, found m/z = 109.03). However, **FU_{58}-3b** was not observed in this MS2 experiment. Finally, **FU_{58}-3b** was clearly observed in enhanced product ion (EPI) MS targeting m/z = 306.20 (Figure S6c) but not in the EPI MS targeting m/z = 198.00 (Figure S6d). This indicates **FU_{58}-3b** is not a decomposition fragment of **FU_{58}-3** but instead an impurity of the obtained substance. As a side note, 4-(methylthio)benzylamine (calcd. for $C_8H_{12}NS$ $[M+H]^+$ 154.07), which could partially have matched the observed electron density, was not observed in any of the MS experiments. Thus, only **FU_{58}-3b** was unambiguously attributed to the electron density and built into the crystal structure obtained by soaking **FU_{58}-3** (PDB ID: 5SAQ).

Table S4. Retrospective, unbiased docking of follow-up compounds.

Starting Fragment	Follow-Up	fragment core at similar position as starting fragment?	# of FlexX poses	successfully HYDE-scored poses	HYDE-scored poses without bad clashes/ torsions	RMSD to crystallographic pose < 2 Å		
						total	in top 10 scored	in top3 scored
F005	**FU_5-1**	yes	500	405	403	59	0	0
	FU_5-2	yes	500	450	442	68	0	0
	FU_5-3	yes	467	413	412	71	0	0
	FU_5-4	no	448	381	378	0	0	0
F058	**FU_{58}-1**	no	235	125	113	0	0	0
	FU_{58}-2	no	403	319	287	0	0	0
F066	**FU_{66}-1**	no	333	202	127	0	0	0
F290	**FU_{290}-1**	yes	386	343	236	0	0	0
	FU_{290}-2	yes	500	440	398	25	3	0

Supplementary references

Biitseva, A., Groth, U. & Hordiyenko, O. (2013). *J. Heterocyclic Chem.* **50,** 1140-1145

Wolf, W. & Vollmann, H. (1956). *German Patent* DE 941845

Krimmer, S. G. & Klebe, G. (2015). *J. Comput. Mol. Des.* **29**, 867–883.