Supporting Information

Selective inhibitors of Cyclin-G associated kinase (GAK) as anti-hepatitis C agents

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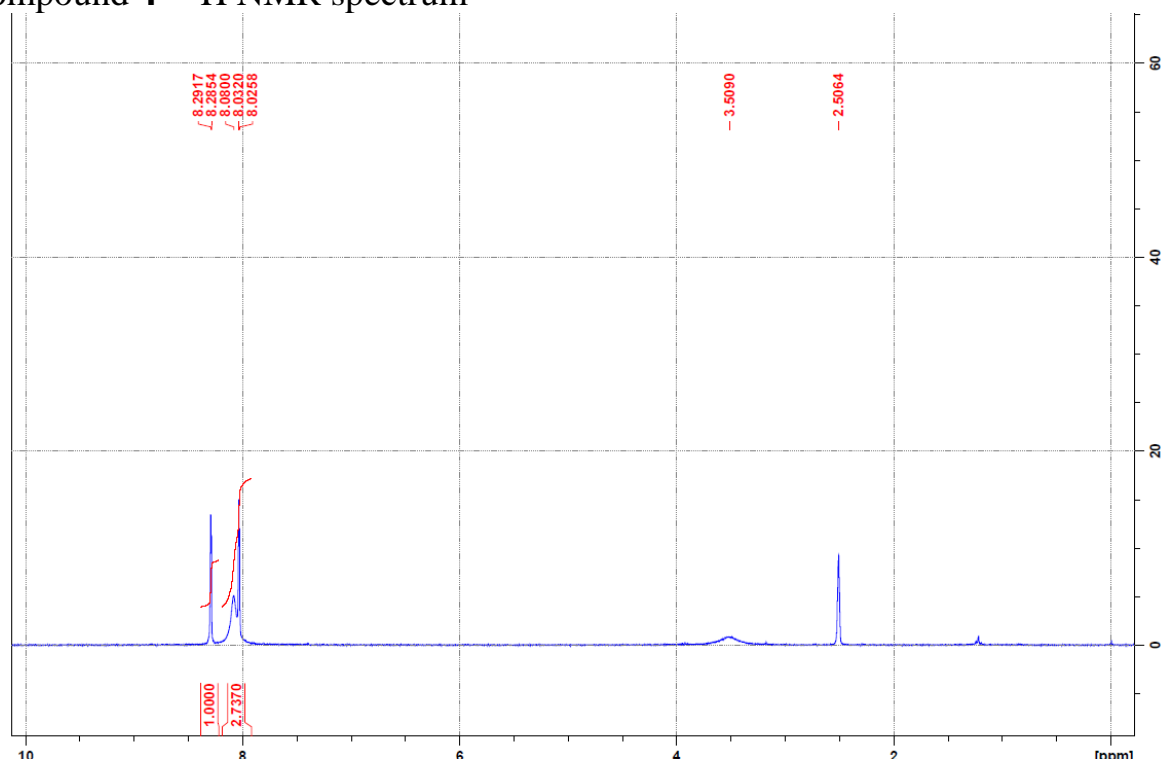
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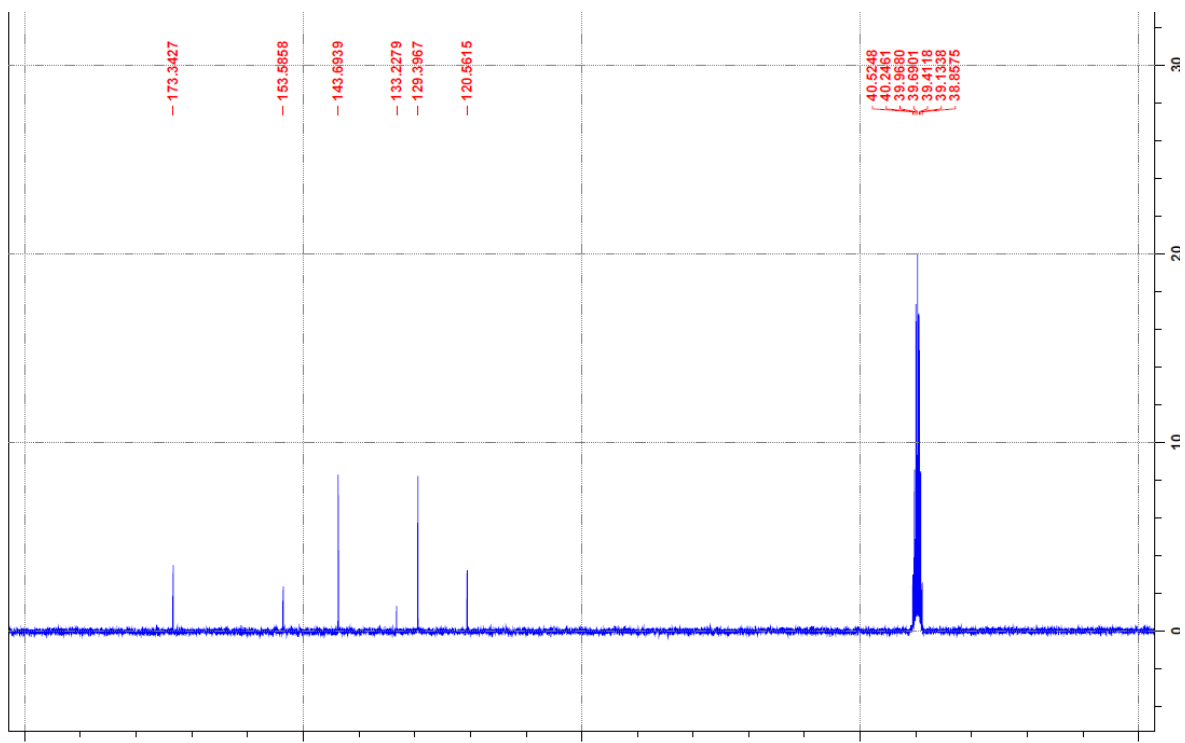
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NMR spectra of selected intermediates and final compounds

Compound **4** - ^{1}H NMR spectrum

Compound **4** – ^{13}C NMR spectrum

Compound **6** – ^{1}H NMR spectrum
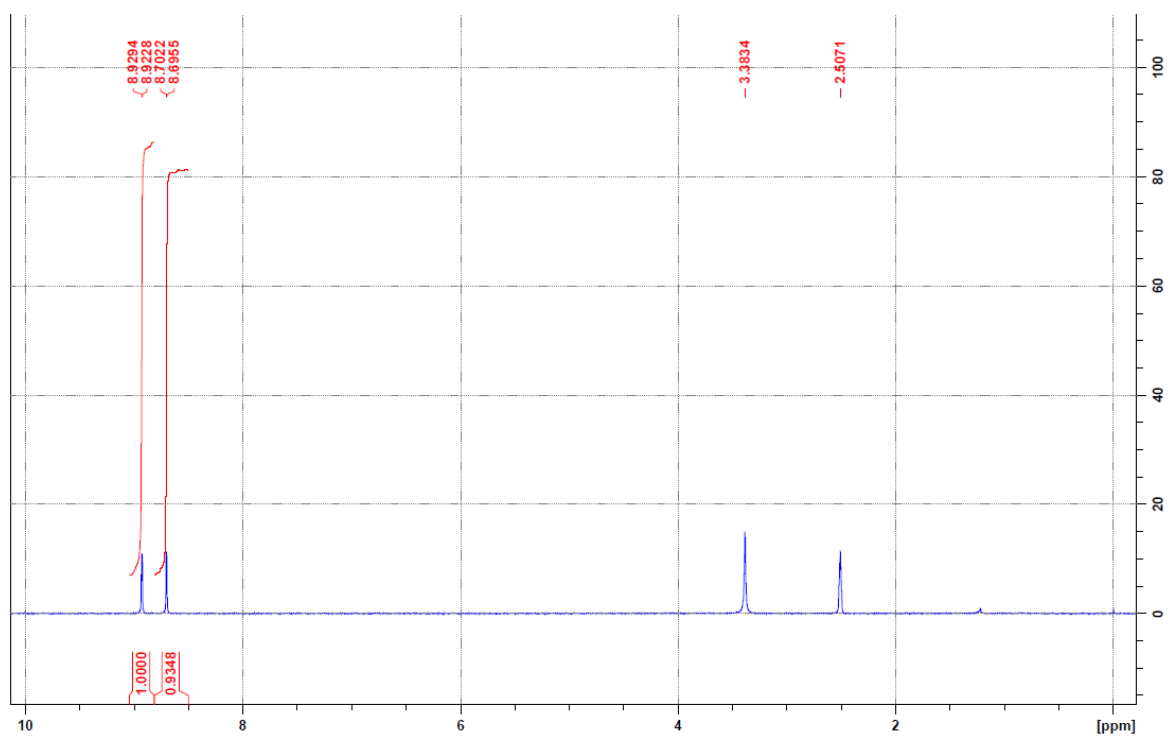
Compound **6** – ^{13}C NMR spectrum
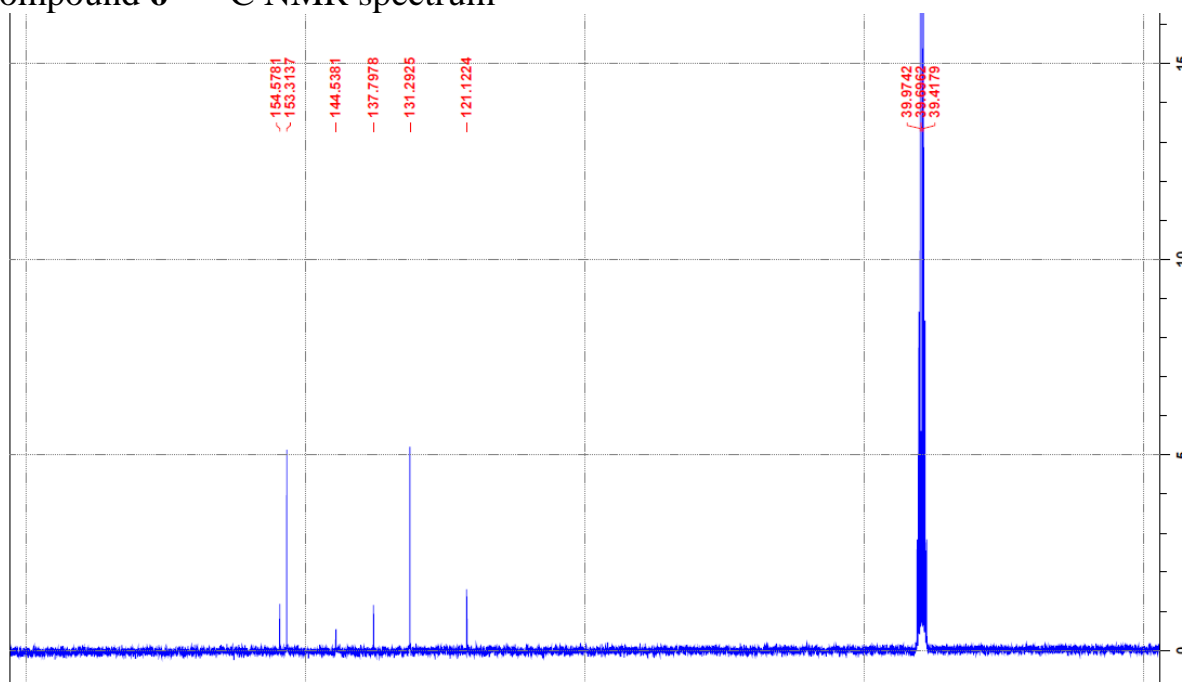
Compound **12g** – ^{1}H NMR spectrum

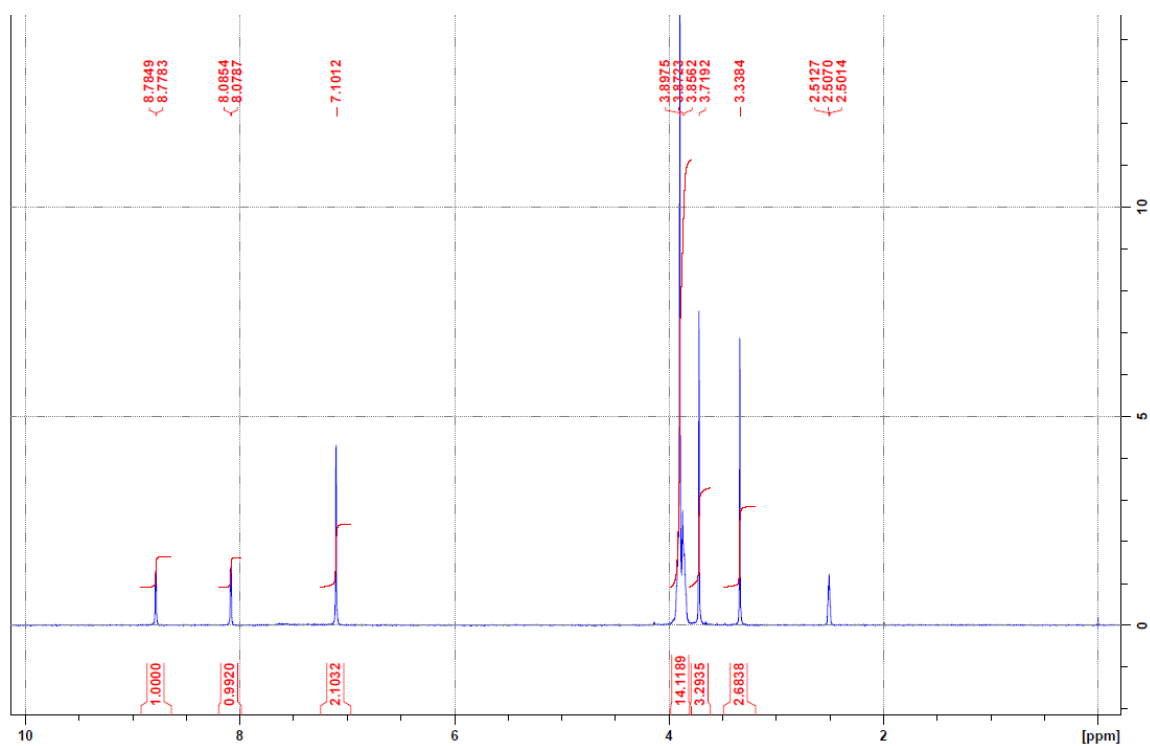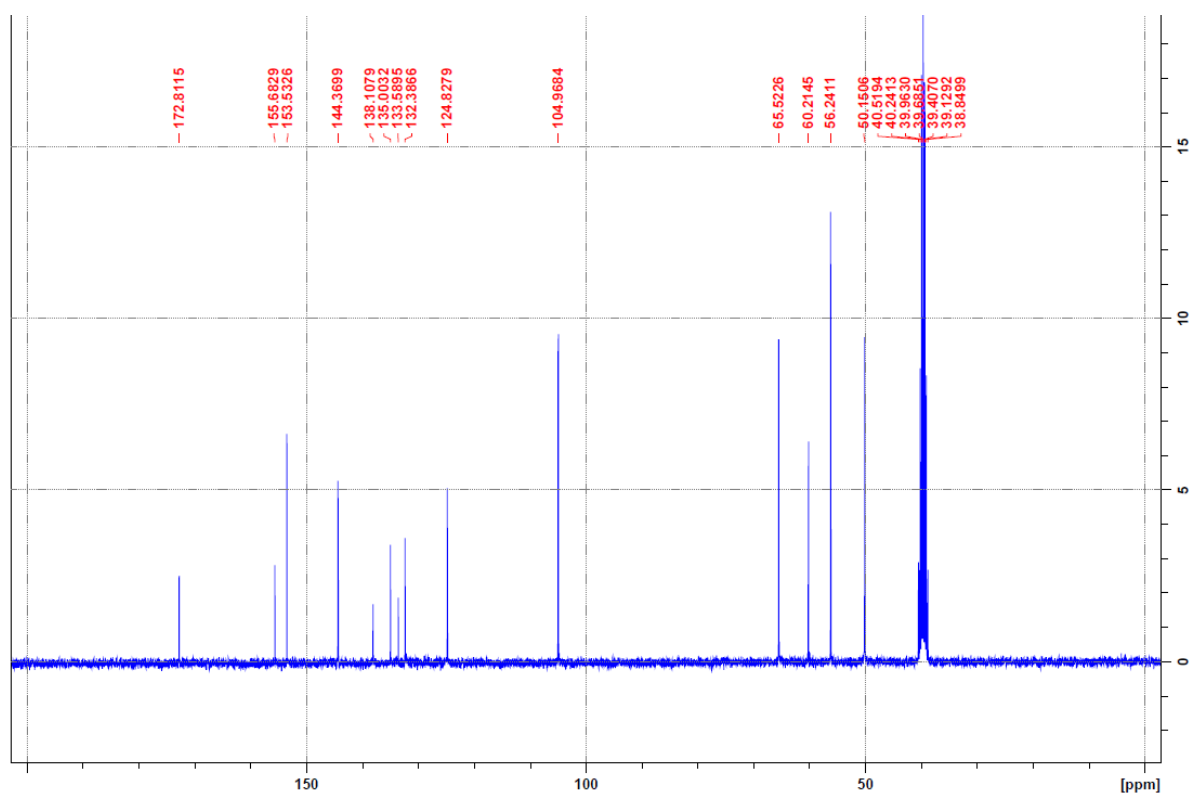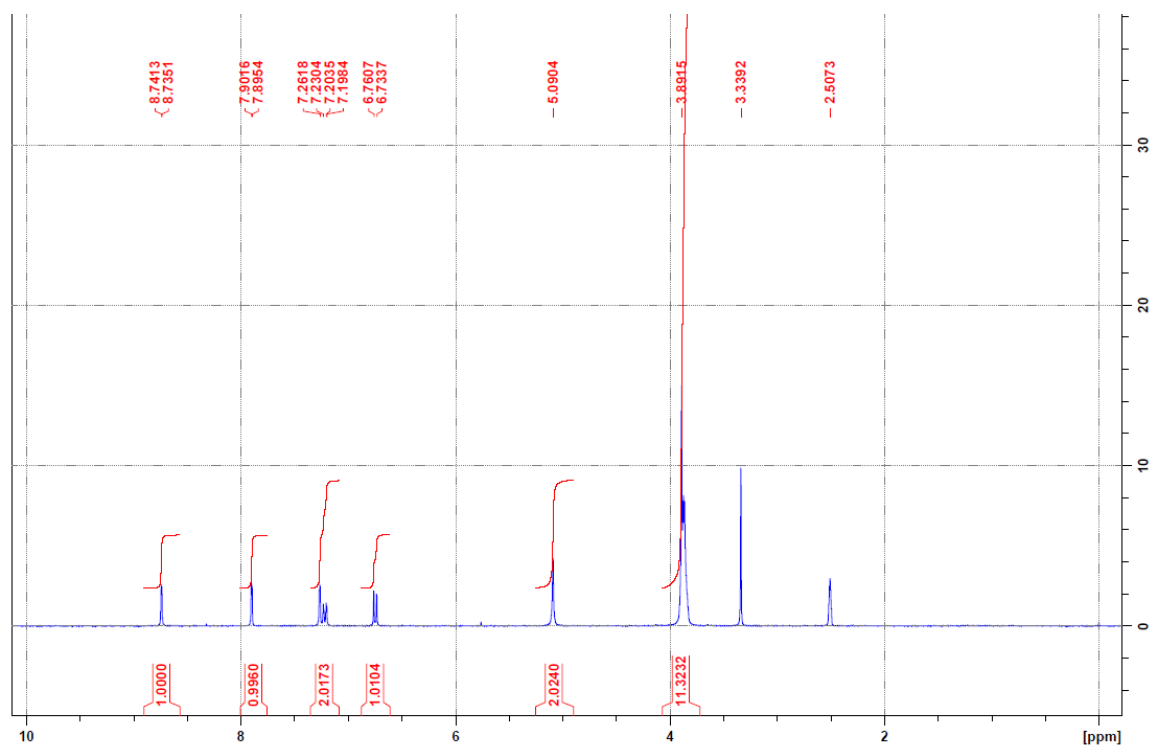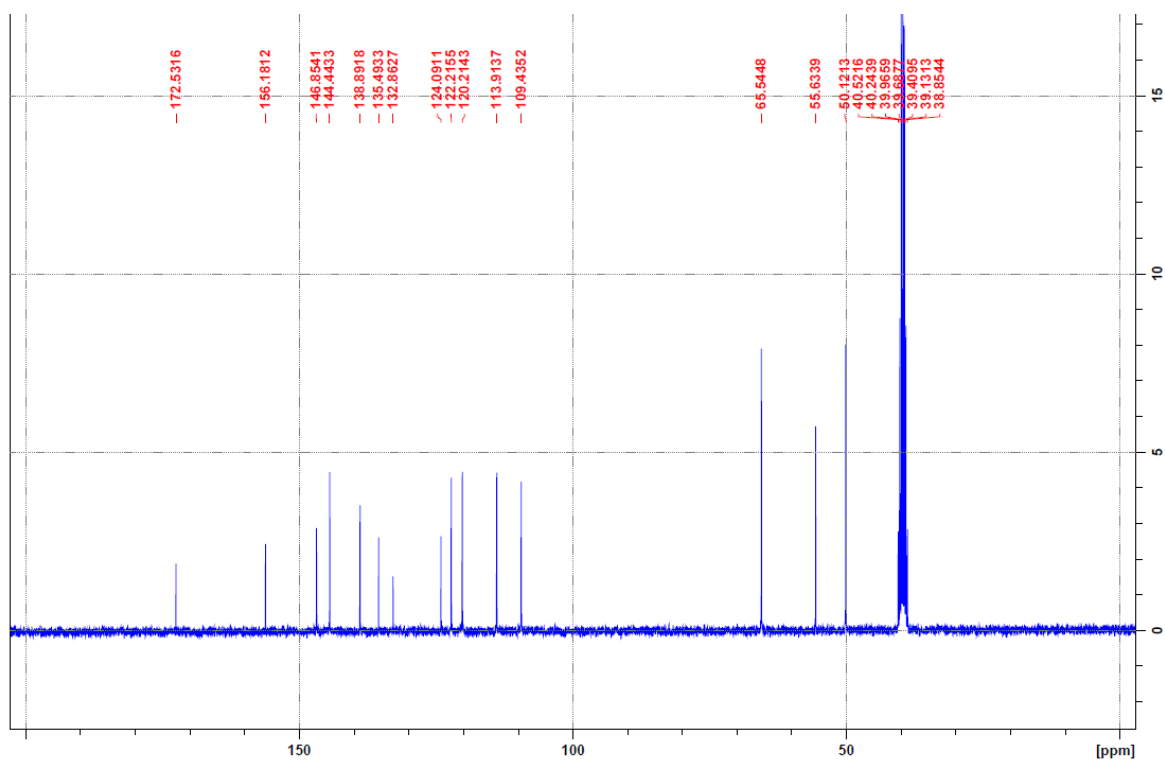
Compound **12g** – ^{13}C NMR spectrum

Compound **12i** – ^{1}H NMR spectrum

Compound **12i** – ^{13}C NMR spectrum

HPLC purity data of final compounds

Purity of final compounds was determined by analytical RP-HPLC analysis on a XBridge column (C-18, 5 μm, 4.6 mm × 150 mm) in combination with a Waters 600 HPLC system and a Waters 2996 photodiode array detector from Waters, Milford, Massachusetts, USA. Elution was done using a gradient mixture of H_2O containing 0.2% (vol) of TFA (A) and Acetonitrile (B) as indicated in Table S2.

Table S1: Gradient table for RP-HPLC purity check.

	Time (min)	Flow (mL/min)	%A	%B
1	0	1	100	0
2	20	1	0	100
3	30	1	0	100
4	31	1	100	0
5	34	1	100	0

Compound#	Rf (min)	Purity (%)
5a	19.245	99.24
5b	18.343	97.37
5c	17.740	99.83
5d	18.242	98.25
5e	15.366	99.89
8a	18.229	96.96
8b	18.291	98.54
8c	18.181	99.89
8d	17.710	98.87
8e	15.508	99.94
9a	22.799	99.93
9b	22.981	99.97
9c	21.270	100
9d	22.016	99.46
9e	16.641	96.88
10a	18.413	99.89
10b	18.836	96.68
10c	17.888	99.79
10d	18.325	99.52
10e	15.423	99.58
11a	21.868	99.69
11b	16.116	90.32
11c	21.681	100
11d	21.045	98.83
11e	22.126	100
12a	24.15	99.78
12b	21.78	99.27
12c	24.23	99.46
12d	24.76	99.43
12e	24.83	97.93
12f	23.21	98.17
12g	24.13	99.88
12h	23.70	95.96
12i	18.25	95.19
12j	21.62	98.58

13a	20.983	99.37
13b	19.44	99.77
13c	18.64	100
13d	20.46	98.75
13e	21.86	98.12
13f	22.529	99.45
13g	23.347	96.68
13h	23.622	99.89
13i	20.49	95.48
13j	20.68	97.14
13k	22.94	96.93

X-ray crystallography

Table S2: Data collection and refinement statistics

Complex	GAK:NbGAK_4-12i
PDB accession code	4Y8D
Data Collection	
Beamline	Diamond, beamline I04-1
Wavelength (Å)	0.9200
Resolution[a] (Å)	43.58-2.10 (2.21-2.10)
Spacegroup	*P* 1
Cell dimensions	$a = 37.7, b = 68.7, c = 89.9$ Å
	$\alpha = 109.7, \gamma = 95.1°, \beta = 99.0°$
No. unique reflections[a]	45,797 (6,533)
Completeness[a] (%)	94.7 (92.4)
I/σI[a]	11.9 (2.0)
R_{merge}[a]	0.071 (0.602)
Redundancy[a]	3.7 (3.6)
Wilson B-factor (amplitudes, Å^2)	42.9
Refinement	
ligands	Compound **12i**
No. atoms in refinement (P/L/O)[b]	5,975/ 48/ 418
R_{fact} (%)	19.2
R_{free} (%)	23.2
B-factors (P/L/O)[b] (Å^2)	72/ 47/ 46
rms deviation bond[c] (Å)	0.014
rms deviation angle[c] (°)	1.4
Error in coordinates by Luzzati (Å)	0.34
Molprobity	
Ramachandran favour	97.0
Ramachandran allowed	100.0

[a] Values in brackets show the statistics for the highest resolution shells.
[b] P/L/O indicate protein, ligand molecule, and other (water and solvent molecules), respectively.
[c] rms indicates root-mean-square.

Supplemental Figure 1: B-factor distribution of the GAK catalytic domain as well as the nanobody. B-factors have been rendered according to colour (as indicated in the figure) and radii of the backbone trace (with increasing radii reflecting increasing B-values).