--Supporting Information--

Is NMR fragment screening fine-tuned to assess the druggability of protein-protein interactions?

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Contents:

1 - Protein expression and purification

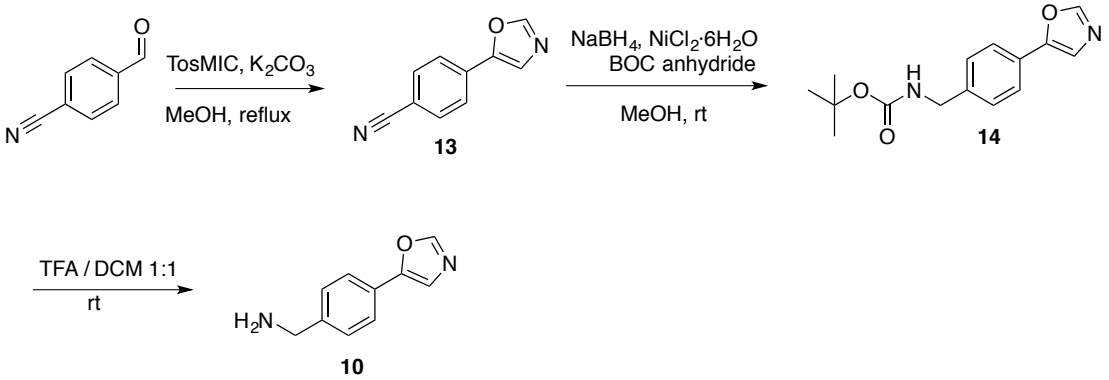
The pVHL:ElonginC:ElonginB (VCB) complex for biophysical and crystallization studies was obtained as previously described. [1,2]
For ^{15}N fully-labeled VCB protein preparation, the following minimal medium was used: 6 g/L Na_2HPO_4, 3 g/L KH_2PO_4, 0.5 g/L NaCl, 2 g/L ^{15}N-NH_4Cl, 3 g/L D-Glucose, 0.011 g/L $CaCl_2$, 0.12 g/L $MgSO_4$, 2 mL/L of 1000× trace elements stock solution (0.5 g/L $CaCl_2{\cdot}2H_2O$, 16.7 mg/L $FeCl_3{\cdot}6H_2O$, 0.18 g/L $CuSO_4{\cdot}5H_2O$, 0.11 g/L $MnSO_4{\cdot}H_2O$, 0.18 g/L $CoCl_2{\cdot}6H_2O$ and 20.1 g/L EDTA). Cultures were inoculated with 1:40 v/v of steady state seed culture, grown at 37°C to an OD_{600} of 0.9 and induced with 0.6 mM IPTG overnight at 24°C. Afterwards, the VCB complex was purified in the same way as unlabeled protein. The final yield was ~64 mg of ^{15}N-VCB from 6 liters of the culture (~11mg per liter).

2 - Chemicals and synthesis

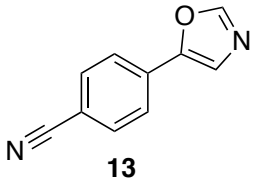
All reagents and solvents were obtained from commercial sources, and used as supplied unless otherwise indicated. Reactions requiring anhydrous conditions were conducted in heated glassware (heat gun), under an inert atmosphere (argon), and using anhydrous solvents. CH_2Cl_2, toluene and MeOH were distilled over CaH_2. Et_2O was distilled on $CaH_2/LiAlH_4$. All reactions were monitored by analytical thin-layer chromatography (TLC) using indicated solvent systems on E. Merck silica gel 60 F254 plates (0.25 mm). TLC plates were visualized using UV light (254 nm) and/or by staining in potassium permanganate followed by heating. Solvents were removed by rotary evaporator below 40˚C and the compounds further dried using high vacuum pumps.

^1H and ^{13}C NMR were recorded on a Bruker Advance 400 MHz and 100 MHz NMR spectrometer respectively. Chemical shifts (δ H) are quoted in ppm (parts per million) and referenced to residual solvent signals: ^1H δ = 7.26 ($CDCl_3$), 2.50 (d_6-DMSO), 3.31 (CD_3OD), ^{13}C δ = 77.0 ($CDCl_3$), 39.43 (d_6-DMSO), 49.05 (CD_3OD). Coupling constants (*J*) are given in Hz. High resolution mass spectra (ESI+) were recorded on a Waters LCT Premier Mass Spectrometer.

10:



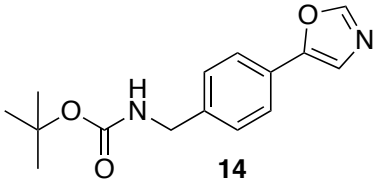
4-(Oxazol-5-yl)benzonitrile, 13



13

13 was synthesized as described previously [2]

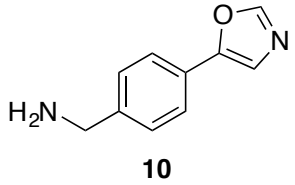
***tert*-Butyl 4-(oxazol-5-yl)benzylcarbamate, 14**



14

14 was synthesized as described previously [2]

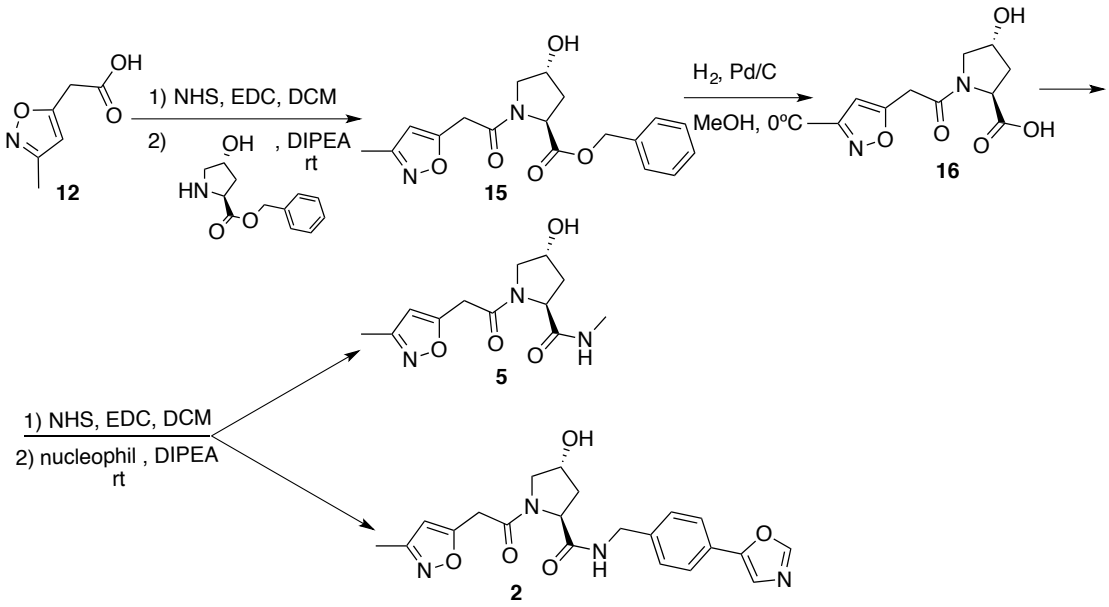
(4-(Oxazol-5-yl)phenyl)methanamine, 10



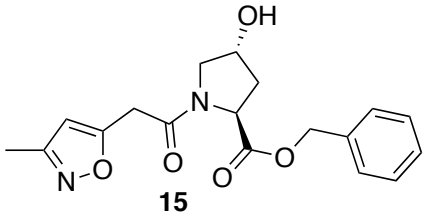
10

10 was synthesized as described previously [2]

2 / 5:



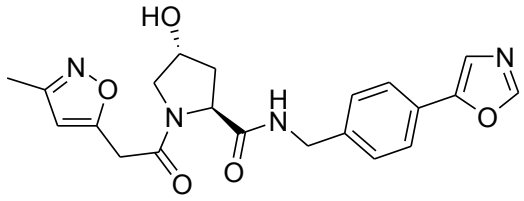
(2*S*,4*R*)-benzyl-4-hydroxy-1-(2-(3-methylisoxazol-5-yl)acetyl)pyrrolidine-2-carboxylate, 15



15

To a room temperature suspension of 2-(3-methylisoxazol-5-yl)acetic acid, **12** (1.0 g, 7.09 mmol, 1 eq.) in freshly distilled CH_2Cl_2 (30 mL), under inert atmosphere (argon), were successively added *N*-hydroxysuccinimide (1.06 g, 9.21 mmol, 1.3 eq) and *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (1.49 g, 7.80 mmol, 1.1 eq.). The resulting mixture was stirred at rt for 4.5 hours. (2*S*,4*R*)-benzyl 4-hydroxypyrrolidine-2-carboxylate hydrochloride (2.01 g, 7.80 mmol, 1.1 eq) and *N*,*N*-diisopropylethylamine (2.59 mL, 14.9 mmol, 2.1 eq.) were then added, and the resulting mixture was stirred at rt for 18 hours. The organic phase was washed with saturated aqueous $NaHCO_3$ (50 mL) and the phases were separated. The aqueous phase was extracted further with $CHCl_3$ (3 x 50 mL). The combined organic phases were dried ($MgSO_4$) and concentrated *in vacuo*. The product **15** (1.85 g, 76%) was obtained as an amorphous off white solid after flash column chromatography (100% AcOEt). R_f 0.3 (AcOEt); ^{1}H NMR (400 MHz, $CDCl_3$, major rotamer) δ 2.00-2.07 (m, 1H), 2.20-2.32 (m, 4H), 3.16 (br. s, 1H), 3.57 (m, 1H), 3.70 (dd, *J* = 10.9, 4.6 Hz, 1H), 3.77 (s, 2H), 4.50 (m, 1H), 4.64 (app-t, *J* = 8.2 Hz, 1H), 5.11 (d, *J* = 12.2 Hz, 1H), 5.18 (d, *J* = 12.2 Hz, 1H), 6.10 (s, 1H), 7.29-7.36 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$, major rotamer) δ 11.4, 33.3, 37.6, 55.6, 58.0, 67.0, 70.0, 104.1, 128.1, 128.3, 128.5, 135.4, 160.2, 165.5, 166.1, 171.7. HRMS (ESI+) m/z calc. for $C_{18}H_{21}N_2O_5$ $[M+H]^+$ 345.1445, found: 345.1474.

(2*S*,4*R*)-4-hydroxy-1-(2-(3-methylisoxazol-5-yl)acetyl)-N-(4-(oxazol-5-yl)benzyl)pyrrolidine-2-carboxamide, 2



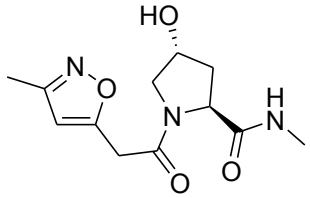
2

To a solution of (2*S*,4*R*)-benzyl 4-hydroxy-1-(2-(3-methylisoxazol-5-yl)acetyl)pyrrolidine-2-carboxylate, **15** (700 mg, 2.03 mmol, 1 eq.) in freshly distilled and degassed (bubbling with argon) MeOH (9.8 mL) at 0°C and under inert atmosphere (argon), was added 10% palladium on charcoal (107 mg, 0.102 mmol, 0.05 eq.). The resulting mixture was saturated with hydrogen (bubbling) and was stirred for 1.25 hour at 0°C under hydrogen atmosphere. The mixture was then degassed (bubbling with argon), filtered on celite and concentrated *in vacuo*. (2*S*,4*R*)-4-hydroxy-1-(2-(3-methylisoxazol-5-yl)acetyl)pyrrolidine-2-carboxylic acid, **16** was obtained as a white solid and used for the next step without further purification. A

small sample was purified by reverse phase HPLC (Gilson GX-271) for analysis and biophysical studies: Pursuit XRs C_{18} (5μm; 250 21.2 mm)(Agilent), 5:95 to 60:40 MeCN/H_2O over 25 minutes, RT = 13minutes. ^{1}H NMR (400 MHz, CD_3OD, major rotamer) δ 2.05-2.15 (m, 1H), 2.25-2.35 (m, 4H), 3.62 (m, 1H), 3.77 (m, 1H), 3.92 (app-s., 2H), 4.48-4.53 (m, 2H), 6.23 (s, 1H). ^{13}C NMR (100 MHz, CD_3OD, major rotamer) δ 11.2, 33.9, 38.8, 56.6, 59.4, 70.9, 105.3, 161.6, 167.6, 168.6, 175.4. HRMS (ESI+) m/z calc. for $C_{11}H_{15}N_2O_5$ $[M+H]^+$ 255.0975, found: 255.0862.

To a room temperature suspension of (2*S*,4*R*)-4-hydroxy-1-(2-(3-methylisoxazol-5-yl)acetyl)pyrrolidine-2-carboxylic acid, **16** (2.03 mmol, 1 eq.) in $CHCl_3$ (14.9 mL), under inert atmosphere (argon), were successively added *N*-hydroxysuccinimide (304 mg, 2.64 mmol, 1.3 eq.) and *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (428 mg, 2.23 mmol, 1.1 eq.). The resulting mixture was stirred at room temperature for 3 hours. (4-(oxazol-5-yl)phenyl)methanamine hydrochloride, **10** (471 mg, 2.23 mmol, 1.1 eq.) and *N,N*-diisopropylethylamine (742 μL, 4.26 mmol, 2.1 eq.) were then added, and the resulting mixture was stirred at room temperature for 24 hours. The product partially precipitated as a white solid. A small amount of MeOH was added for complete solubilisation of the precipitate. The reaction mixture was diluted with $CHCl_3$ and washed with saturated aqueous $NaHCO_3$. The aqueous phase was extracted 3 times with generous amounts of $CHCl_3$. The combined organic phases were dried ($MgSO_4$) and concentrated *in vacuo*. The product (648 mg, 78% from (2S,4R)-benzyl-4-hydroxy-1-(2-(3-methylisoxazol-5-yl)acetyl)pyrrolidine-2-carboxylate, **2**) was obtained as an amorphous white solid after flash column chromatography (gradient 95:5 to 90:10 CH_2Cl_2/MeOH). R_f 0.3 (90:10 CH_2Cl_2/MeOH); ^{1}H NMR (400 MHz, CD_3OD, major rotamer); δ 2.05-2.14 (m, 1H), 2.23-2.31 (m, 4H), 3.63 (m, 1H), 3.81 (m, 1H), 3.92 (d, *J* = 17.1 Hz, 1H), 3.97 (d, *J* = 17.1 Hz, 1H), 4.42 (d, *J* = 15.4 Hz, 1H), 4.48 (d, *J* = 15.4 Hz, 1H), 4.53 (m, 1H), 4.58 (m, 1H), 6.25 (s, 1H), 7.43 (m, 2H), 7.50 (s, 1H), 7.70 (m, 2H), 8.25 (s, 1H). ^{13}C NMR (100 MHz, CD_3OD, major rotamer) δ 11.3, 34.0, 39.4, 43.8, 57.0, 60.8, 70.9, 105.4, 121.9, 125.7, 127.8, 129.1, 140.8, 152.8, 153.2, 161.6, 167.7, 168.8, 174.5. HRMS (ESI+) m/z calc. for $C_{21}H_{23}N_4O_5$ $[M+H]^+$ 411.1663, found: 411.1705.

(2*S*,4*R*)-4-hydroxy-N-methyl-1-(2-(3-methylisoxazol-5-yl)acetyl)pyrrolidine-2-carboxamide, 5

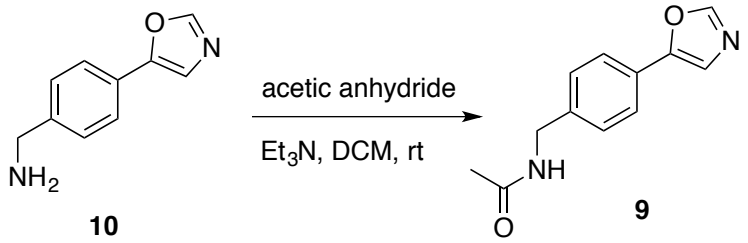


5

To a room temperature suspension of (2*S*,4*R*)-4-hydroxy-1-(2-(3-methylisoxazol-5-yl)acetyl)pyrrolidine-2-carboxylic acid, **16** (0.48 mmol, 1 eq.) in $CHCl_3$ (3.2 mL) , under inert atmosphere (argon), were successively added *N*-hydroxysuccinimide (65 mg, 0.57 mmol, 1.3 eq.) and *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (92 mg, 0.48 mmol, 1.1 eq.). The resulting mixture was stirred at room temperature for 3 hours. Methylamine (8M in EtOH, 82 μL, 0.65 mmol, 1.5 eq.) and *N,N*-diisopropylethylamine (91 μL, 0.52 mmol, 1.2 eq.) were then

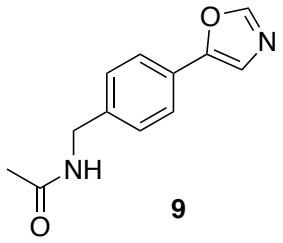
added, and the resulting mixture was stirred at room temperature for 24 hours. The reaction mixture was concentrated *in vacuo* and the product, **5** (64 mg, 55% from (2*S*,4*R*)-benzyl-4-hydroxy-1-(2-(3-methylisoxazol-5-yl)acetyl)pyrrolidine-2-carboxylate, **15**) was obtained as an amorphous white solid after flash column chromatography (gradient 95:5 to 90:10 CH_2Cl_2/MeOH). R_f 0.25 (90:10 CH_2Cl_2/MeOH); ^{1}H NMR (400 MHz, CD_3OD, major rotamer); δ 1.98-2.08 (m, 1H), 2.18-2.28 (m, 4H), 2.73 (s, 3H), 3.61 (m, 1H), 3.78 (m, 1H), 3.89 (d, *J* = 17.1 Hz, 1H), 3.93 (d, *J* = 17.1 Hz, 1H), 4.44-4.52 (m, 2H), 6.23 (s, 1H). ^{13}C NMR (100 MHz, CD_3OD, major rotamer) δ 11.3, 26.4, 34.0, 39.4, 57.0, 60.7, 70.9, 105.4, 161.6, 167.7, 168.7, 174.9. HRMS (ESI+) *m/z* calc. for $C_{12}H_{18}N_3O_4$ $[M+H]^+$ 268.1292, found: 268.1302.

9:



10 **9**

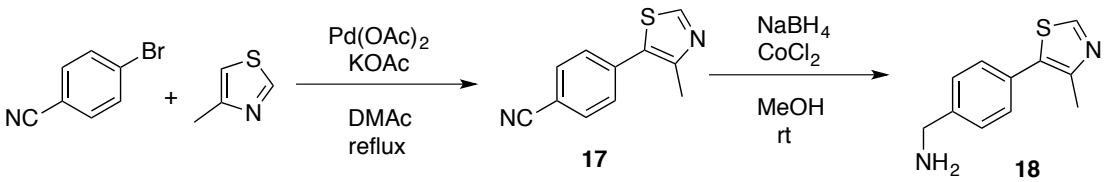
N-(4-(Oxazol-5-yl)benzyl)acetamide, 9



9

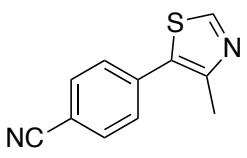
9 was synthesized as described previously [2]

18:



17 **18**

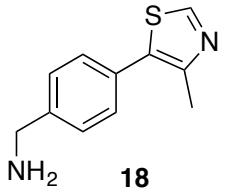
4-(4-methylthiazol-5-yl)benzonitrile, 17



17

17 was synthesized as described previously [3]

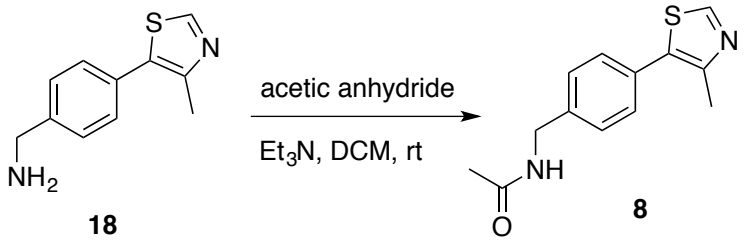
(4-(4-methylthiazol-5-yl)phenyl)methanamine, 18



18

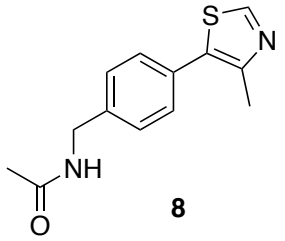
18 was synthesized as described previously [3]

8:



18 **8**

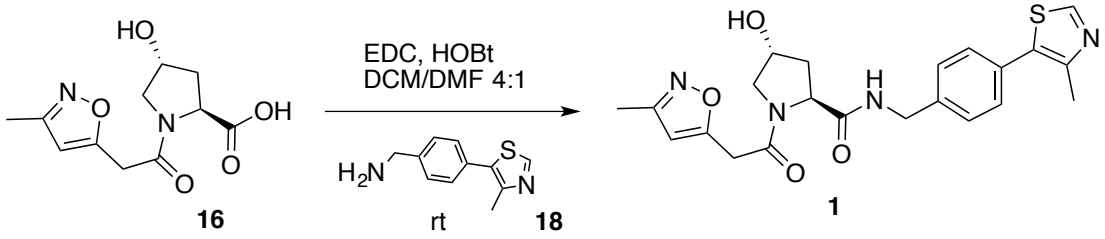
***N*-(4-(4-methylthiazol-5-yl)benzyl)acetamide, 8**



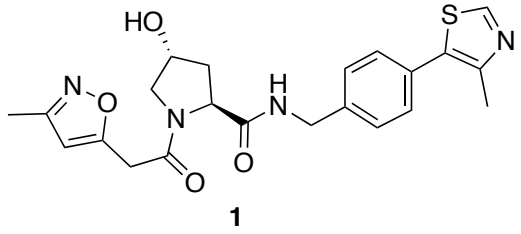
8

To a room temperature suspension of (4-(4-methylthiazol-5-yl)phenyl)methanamine, **18** (100 mg, 0.49 mmol, 1 eq.) in CH_2Cl_2 (6 mL) under inert atmosphere (argon), was added triethylamine (147 μL, 107 mg, 1.06 mmol). The resulting mixture was stirred at room temperature for 10 minutes. Anhydride acetic was added dropwise and the resulting mixture was stirred at room temperature for 18 hours. The reaction was quenched with 10% HCl (5 mL) and the aqueous layer was extracted with CH_2Cl_2 (3x10 mL). The combined organic layers were dried ($MgSO_4$) and concentrated *in vacuo* to give the product **8** (99 mg, 82% isolated) as a yellow pale solid after flash column chromatography (gradient 100 CH_2Cl_2 to 97:3 CH_2Cl_2/MeOH). R_f 0.73 (90:10 CH_2Cl_2/MeOH). ^{1}H NMR (400 MHz, CD_3OD) δ 2.00 (s, 3H), 2.47 (s, 3H), 4.40 (s, 2H), 7.38-7.45 (m, 4H), 8.87 (s, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ 15.7, 22.5, 43.8, 129.1, 130.5, 131.7, 133.3, 140.4, 149.1, 151.2, 173.2. HRMS (ESI+) *m/z* calc. for $C_{13}H_{15}N_2OS$ $[M+H]^+$ 247.0905, found: 247.0901.

1:

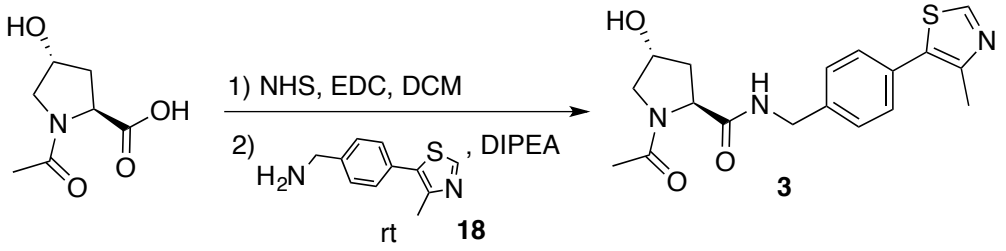


(2*S*,4*R*)-4-hydroxy-1-(2-(3-methylisoxazol-5-yl)acetyl)-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide, 1

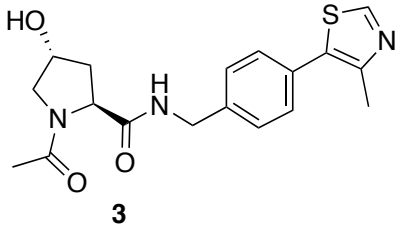


To a room temperature suspension of (2*S*,4*R*)-4-hydroxy-1-(2-(3-methylisoxazol-5-yl)acetyl)pyrrolidine-2-carboxylic acid, **16** (66 mg, 0.25 mmol), 1 eq. in CH_2Cl_2:DMF 4:1 (3 mL) under inert atmosphere (argon), were successively added 1-hydroxybenzotriazole (60 mg, 0.39 mmol, 1.5 eq.) and *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (65 mg, 0.34 mmol, 1.3 eq.). The resulting mixture was stirred at room temperature for 5 minutes. (4-(4-methylthiazol-5-yl)phenyl)methanamine, 1**8** (54 mg, 0.26 mmol, 1 eq.) dissolved in in CH_2Cl_2:DMF 4:1 (2 mL) was added. Upon stirring for 15 h the reaction was diluted with 10 mL CH_2Cl_2, and washed with 15 mL sat. $NaHCO_3$ (x3). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* to give the product **1** (49 mg, 43% isolated) as a white solid after flash column chromatography (gradient 97:3 to 95:5 CH_2Cl_2/MeOH) that matched the reported spectral data.[3] R_f 0.46 (90:10 CH_2Cl_2/MeOH) HRMS (ESI+) *m/z* calc. for $C_{22}H_{24}N_4O_4S$ $[M+H]^+$ 441.1597, found: 441.1632.

3:



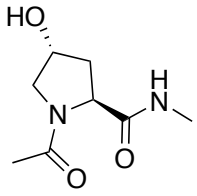
(2*S*,4*R*)-1-acetyl-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide, 3



3

To a room temperature suspension of (2*S*,4*R*)-1-acetyl-4-hydroxypyrrolidine-2-carboxylic acid (200 mg, 1.15 mmol, 1 eq.) in CH_2Cl_2 (3 mL), under inert atmosphere (argon), were successively added *N*-hydroxysuccinimide (172 mg mg, 1.50 mmol, 1.3 eq.) and *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (241 mg, 1.26 mmol, 1.1 eq). The resulting mixture was stirred at room temperature for 3 hours. (4-(4-methylthiazol-5-yl)phenyl)methanamine, **18** (283 mg, 1.38 mmol, 1.2 eq.) dissolved with CH_2Cl_2 (2 mL) and *N*,*N*-diisopropylethylamine (91 μL, 0.523 mmol, 1.2 eq.) were then added, and the resulting mixture was stirred at room temperature for 18 hours. The reaction mixture was diluted with CH_2Cl_2 (5 mL) and washed with saturated aqueous $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (3x10 mL). The combined organic phases were dried ($MgSO_4$) and concentrated *in vacuo* to give the product **3** (133 mg, 32% isolated) as a white solid after flash column chromatography (gradient 97:3 to 95:5 CH_2Cl_2/MeOH). R_f 0.25 (90:10 CH_2Cl_2/MeOH). ^{1}H NMR (400 MHz, CD_3OD) δ 2.05-2.12 (m, 4H) 2.22-2.30 (m, 1H), 2.48 (s, 3H), 3.55 (d, *J* = 11.2 Hz , 1H), 3.79 (dd, *J* = 4, 4.4 Hz, 1H), 4.35-4.58 (m, 4H), 7.37-7.47 (m, 4H), 8.87 (s, 1H) ^{13}C NMR (101 MHz, CD_3OD, major rotamer) δ 15.8, 22.4, 39.6, 43.6, 57.4, 60.4, 70.8, 128.9, 130.4, 131.3, 140.3, 152.8, 172.6, 174.7. HRMS (ESI+) *m/z* calc. for $C_{18}H_{21}N_3O_3S$ $[M+H]^+$ 360.1382, found: 360.1398.

(2S,4R)-1-Acetyl-4-hydroxy-N-methylpyrrolidine-2-carboxamide, 6



6

6 was synthesized as described previously [1]

3- NMR experiments

All ligand-based NMR experiments were carried out at 278 K using Bruker Avance 500 MHz with TCI cryoprobe. Protein-based ^{1}H-^{15}N 2D NMR HSQC (correlation via double inept transfer, phase sensitive and with decoupling during acquisition) were performed at 305 K on Bruker Avance 700 MHz with TCI cryoprobe (Department of Chemistry, University of Cambridge). ^{1}H-^{15}N 2D NMR HSQC spectra were performed with 64 scans for each of 256 points in indirect dimension and relaxation delay of 1 s that summed up to 5 h of experiment time for each protein spectrum and titration.

All samples for ligand-based NMR experiments were made up to 200 µl in 3 mm capillaries containing 50 mM $NaPO_4$ pH 7.0, 10% (v/v) D_2O and 20 µM (trimethylsilyl)-propionic acid-d_4 (TSP), for calibration purposes. For each set-up three experiments were carried out: 1) control sample – compound alone; 2) + protein sample – protein concentration varies from 10 to 40 µM; and 3) + protein + peptide – same as previous experiment but with the addition of 100 µM HIF-1α 19-mer peptide (DEALA-Hyp-YIPMDDDFQLRSF).
Protein-based NMR samples were made up to 200 µl in 3 mm capillaries in 20 mM Bis-Tris pH 7.0, 150 mM NaCl, 1 mM DTT and using 10% (v/v) D_2O. To titrate compounds into ^{15}N-labeled protein, 100 mM stocks in 100% d_6-DMSO were used in order to have significant less dilution and protein consumption.
NMR experiments are shown in the Figures S1-S13.

4 - X-ray diffraction experiments

The X-ray data collection and statistics for VCB crystals soaked with **2** (pdb code 3zrc) and **6** (pdb code 4awj) have been described previously.[1,2]
In order to collect data for the **4** and **5** bound VCB complexes, VCB crystals were soaked overnight in a 20 mM solution of **4** or **5,** in Na cacodylate pH 6.0, 15% PEG3350, 0.2 M Mg acetate, 5 mM DTT. X-ray data were collected at 100 K at the Diamond synchrotron facilities and processed using XDS. The structures were solved by rigid body refinement using Buster TNT [4] using the VCB apo structure (PDB code 3zrf)[1] as a starting model. The initial structure obtained this way was further refined using Buster TNT and corrected manually using Coot.[5] Data collection statistics and refinement parameters are summarized in **Table S1**.

Table S1. Data collection statistics and refinement parameters for VCB_**4** and VCB_**5** crystal structures

Dataset	**VCB_4**	**VCB_5**
Data collection		
Synchrotron	Diamond Light Source	Diamond Light Source
Beamline	I02	I02
Wavelenght, Å	0.9795	0.9795
Data processing		
Space group	$P4_122$ (91)	$P4_122$ (91)
Cell parameters - a, b, c (Å) ($\alpha=\beta=\gamma=90^o$)	93.66 93.66 365.92	93.66 93.66 365.92
Resolution, Å (outer shell) [(c)]	50.0-2.2 (2.3-2.2)	50.0-2.35 (2.45-2.35)
Total reflections	1073860 (77623)	878250 (64565)
No. of unique reflections	83354 (6032)	68245 (4935)

Completeness	100 (100)	100 (100)
Multiplicity	12.9 (12.9)	12.9 (13.1)
R_{meas}, % (outer shell)	11.8 (163.8)	14.6 (185.2)
$CC_{1/2}$, % (outer shell)	99.9 (81.8)	99.8 (94.0)
$\langle I/\sigma(I)\rangle$ (outer shell)	14.95 (2.18)	15.87 (2.22)
Mosaicity, °	0.09	0.241
Refinement		
Resolution range, Å	46.79-2.20	46.59-2.35
No. of reflections	83335	68204
Percentage observed	99.98	99.98
R_{cryst},[(a)] %	20.66	19.02
R_{free},[(b)] %	24.62	22.75
RMS		
Bonds, Å	1.17	1.19
Angles, °	0.01	0.01
Ramachandran Plot		
Most favored, %	95.42	94.61
Additionally allowed, %	3.34	3.16
Disallowed, %	1.24	2.23
PDB code	4bks	4bkt

[(a)] $R_{cryst} = \sum||F_{obs}|-|F_{calc}||/\sum|F_{obs}|$, F_{obs} and F_{calc} are observed and calculated structure factor amplitudes

[(b)] R_{free} as for R_{cryst} using a random subset of the data excluded from the refinement

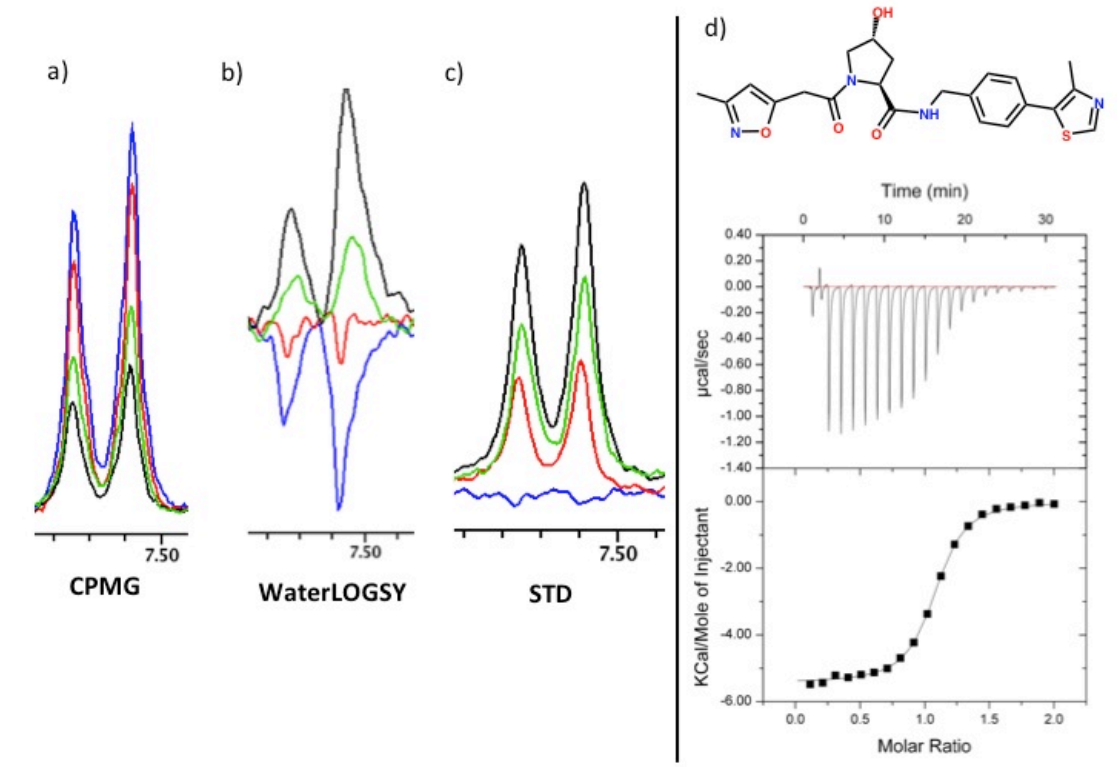
[(c)] Data in brackets are for the highest resolution shell

5 - ITC experiments

ITC experiments were performed using the ITC_{200} instrument from Microcal Inc. (GE Healthcare) at 25°C. VCB protein was in 20 mM Bis-Tris pH 7.0, 150 mM NaCl, 1 mM DTT and loaded into the ITC cell at concentration of 80 μM with the appropriate v/v DMSO solution. Ligands were dissolved in the same buffer/DMSO percentage. Typically, 19 injections of 2.0 μL of ligand solution of 4 s duration at 1 min intervals were performed. An initial injection of ligand (0.5 μL) was made and discarded during data analysis. Data was fitted to single binding site model using the Microcal LLC ITC_{200} Origin software provided by the manufacturer.

ITC traces together with the results of the data fitting are shown in the Figures S1-S13.

6 - Figures



e)

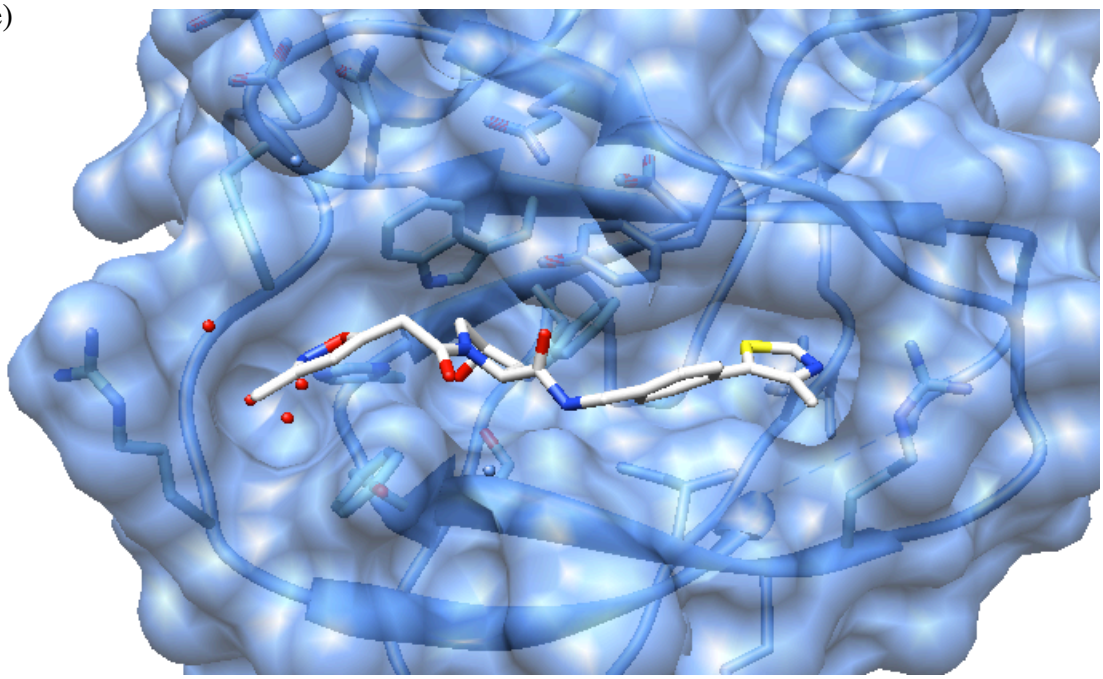
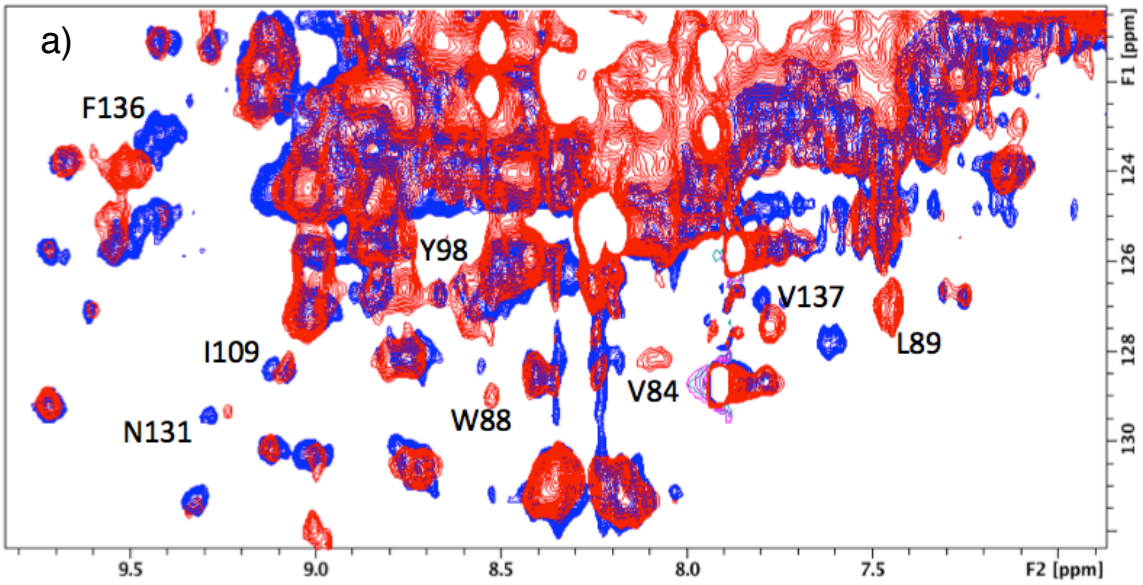


Figure S1: Binding detection using ligand-based NMR spectroscopy for **1**. Panels a), b) and c) depict spectra for VCB+**1** using set-ups 1 and 2 (red and black respectively), the compound alone (blue) and in competition with 100 µM 19-mer HIF-1α peptide under set-up 2 (green). d) Direct ITC titration for **1** (1 mM compound and 100 µM VCB). K_a = 6.3 × 10^5 ± 3.5 × 10^4 M^{-1}; ΔH = –5435 ± 25 cal/mol and ΔS = 8.3 cal/mol/degree. e) Modeled bound **1** (white carbon sticks) into VCB structure. The protein surface is shown in blue at 30% transparency.



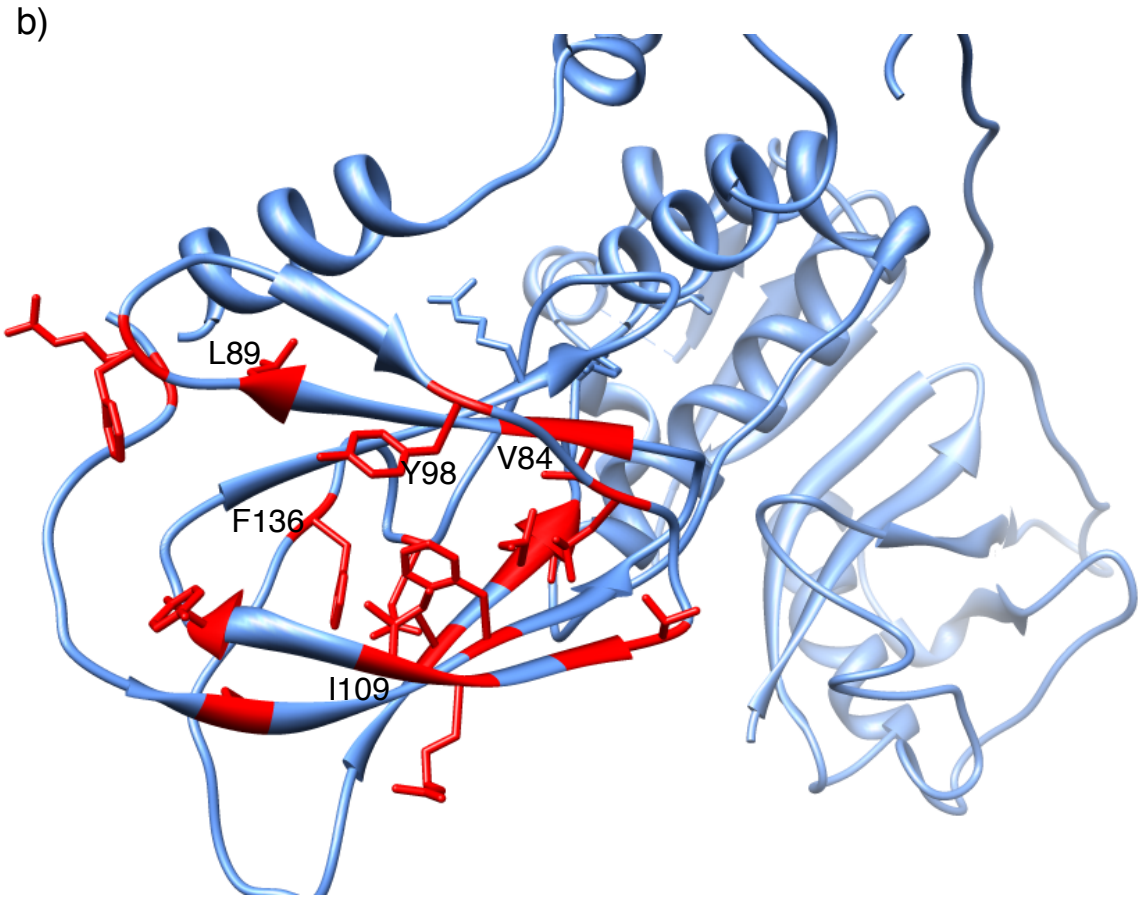


Figure S2: a) Zoom on the 2D ^{1}H-^{15}N HSQC spectrum of 100 µM VCB (red) highlighting the residues shifting due the protein-ligand interaction with 1 mM concentration of **1** (blue). b) Site specific mapping into the VCB structure of the residues involved in the interaction (red).

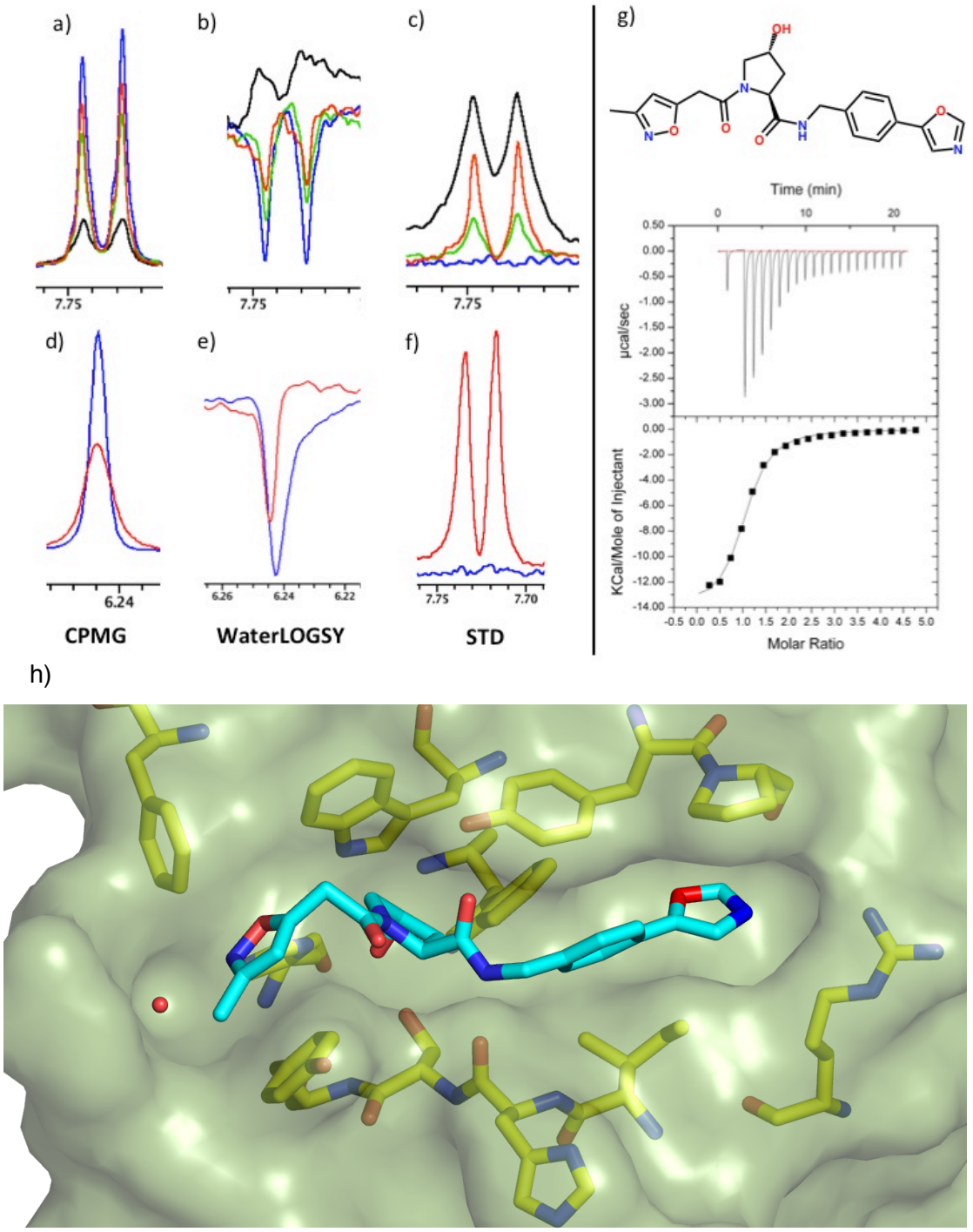


Figure S3: Binding detection using ligand-based NMR spectroscopy for **2**. Panels a), b) and c) depict spectra for VCB+**2** using set-ups 1 and 2 (red and black respectively), the compound alone (blue) and in competition with 100 µM 19-mer HIF-1α peptide under set-up 2 (green). Panels d), e) and f) depict spectra for VCB+**2** using set-up 3 (red) and compound alone (blue). g) Direct ITC titration for **2** (1 mM compound and 100 µM VCB). $K_a = 1.8 \times 10^5 \pm 3.0 \times 10^4$ M^{-1}; $\Delta H = -12150 \pm 671$ cal/mol and $\Delta S = -16.7$ cal/mol/degree. h) Crystal structure of VCB in complex with **2** (cyan carbon sticks).[1] The protein surface is shown in green at 40% transparency.

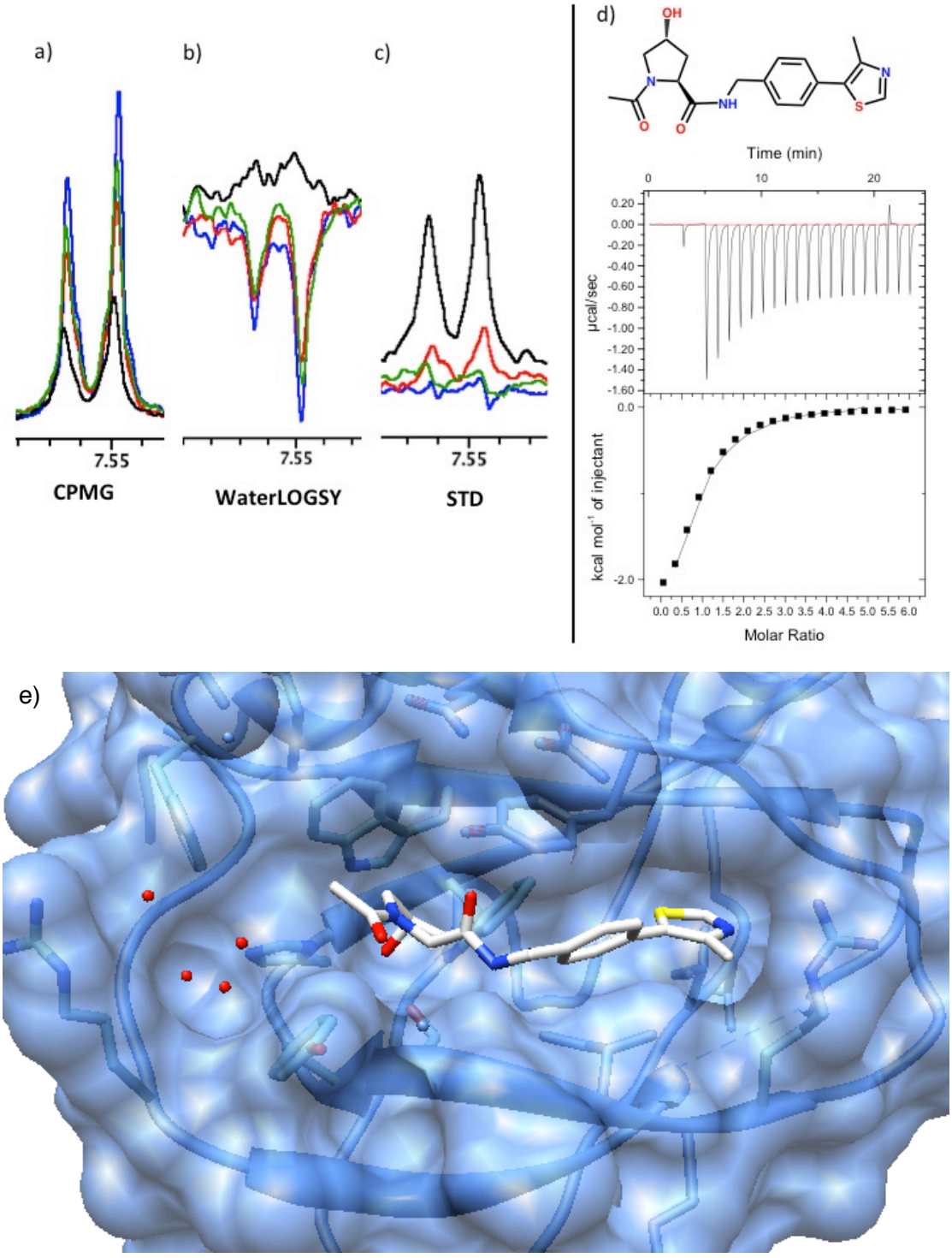


Figure S4: Binding detection using ligand-based NMR spectroscopy for **3**. Panels a), b) and c) depict spectra for VCB+**3** using set-ups 1 and 2 (red and black respectively), the compound alone (blue) and in competition with 100 µM 19-mer HIF-1α peptide under set-up 2 (green). d) Direct ITC titration for **3** (3 mM compound and 100 µM VCB). $K_a = 2.7 \times 10^4 \pm 5.4 \times 10^3$ M^{-1}; $\Delta H = -3200 \pm 472$ cal/mol and $\Delta S = 6.4$ cal/mol/degree. e) Modeled bound **3** (white carbon sticks) into VCB structure. The protein surface is shown in blue at 30% transparency.

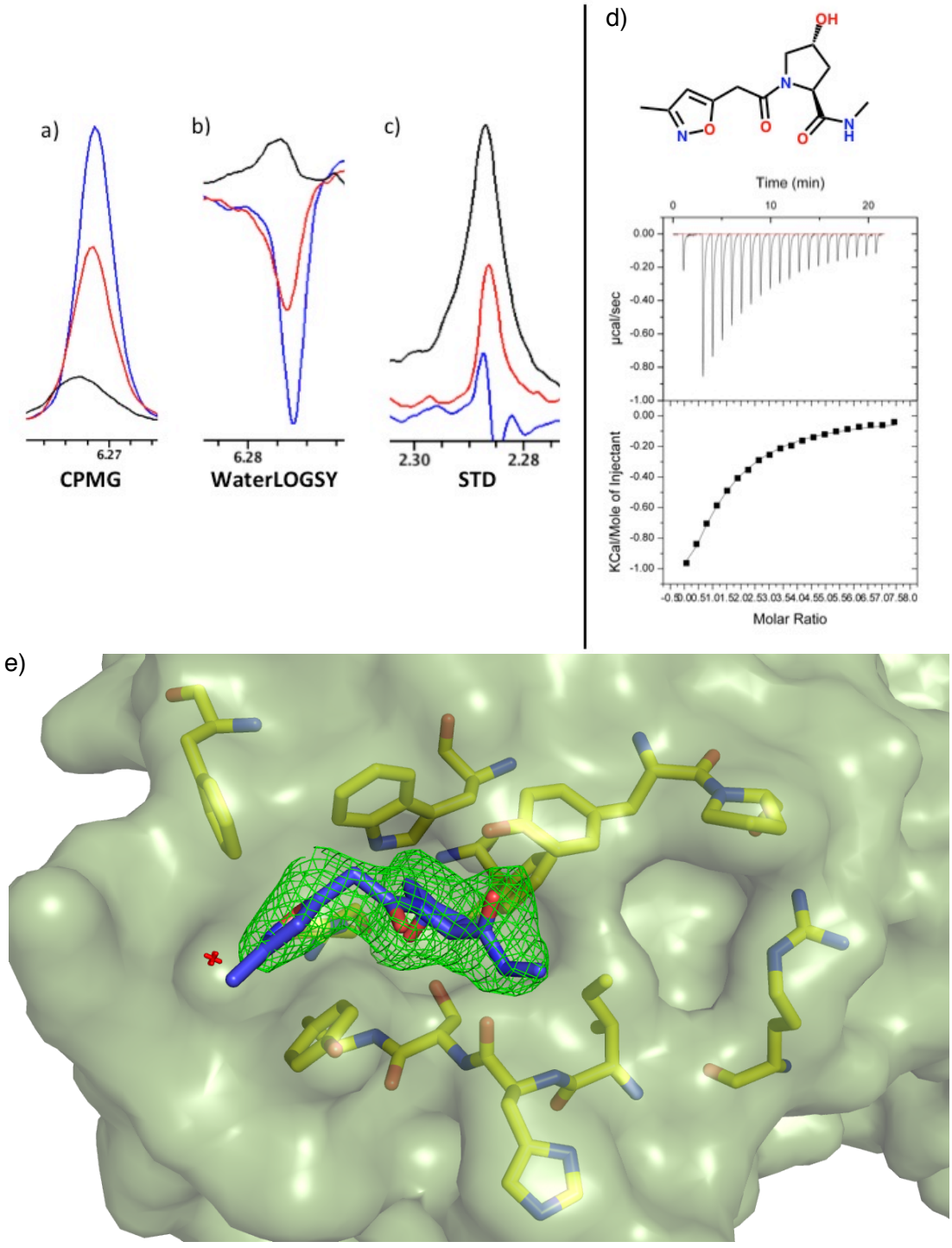


Figure S5: Binding detection using ligand-based NMR spectroscopy for **5**. Panels a), b) and c) depict spectra for VCB+**5** using set-ups 1 and 2 (red and black respectively), the compound alone (blue). d) Direct ITC titration for **5** (3 mM compound and 100 µM VCB). $K_a = 4.17 \times 10^3 \pm 293$ M^{-1}; $\Delta H = -2555 \pm 259$ cal/mol and $\Delta S = 8.0$ cal/mol/degree. e) Crystal structure of VCB in complex with **5** (blue carbon sticks). The omit electron density maps (F_o–F_c) is shown in green contoured at 2.5σ around the ligand. The protein surface is shown in green at 40% transparency.

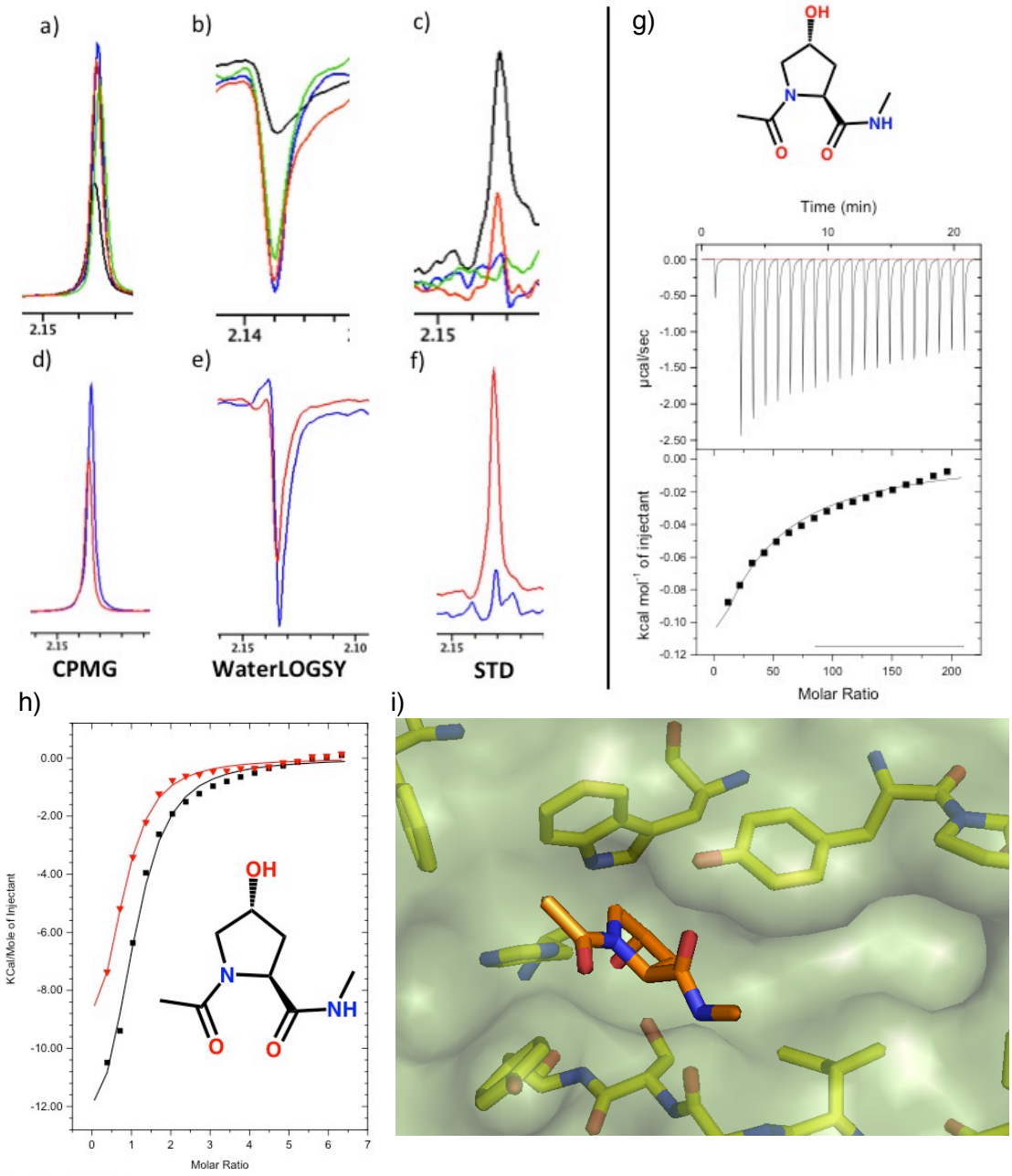


Figure S6: Binding detection using ligand-based NMR spectroscopy for **6**. Panels a), b) and c) depict spectra for VCB+**6** using set-ups 1 and 2 (red and black respectively), the compound alone (blue) and in competition with 100 µM 19-mer HIF-1α peptide under set-up 2 (green). Panels d), e) and f) depict spectra for VCB+**6** using set-up 3 (red) and compound alone (blue). g) Direct ITC titration for **6** (60 mM compound and 100 µM VCB). K_a = 204 ± 19.7 M^{-1}; ΔH = –2415 ± 153 cal/mol and ΔS = -16.7 cal/mol/degree. h) Competitive ITC titration between **2** and **6**. Direct titration of **2** into 100 µM VCB is shown in the absence (black trace) and presence (red trace) of 3 mM of **6**. i) Crystal structure of VCB in complex with **2** (orange carbon sticks).[2] The protein surface is shown in green at 40% transparency.

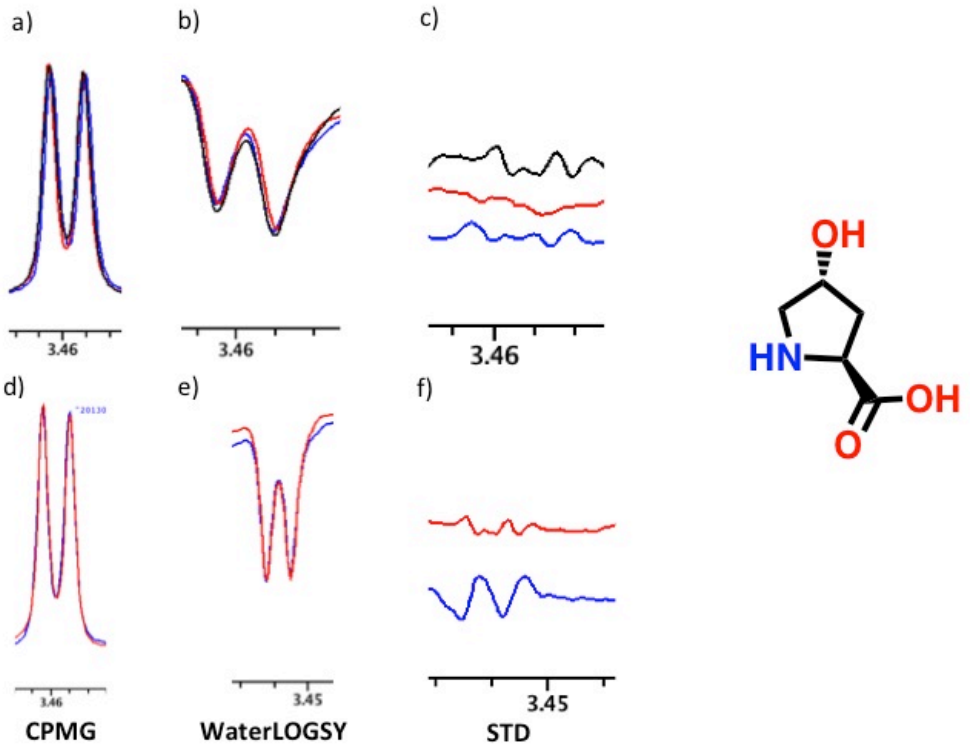


Figure S7: Binding detection using ligand-based NMR spectroscopy for **7**. Panels a), b) and c) depict spectra for VCB+**7** using set-ups 1 and 2 (red and black respectively) and the compound alone (blue). Panels d), e) and f) depict spectra for VCB+**7** using set-up 3 (red) and compound alone (blue).

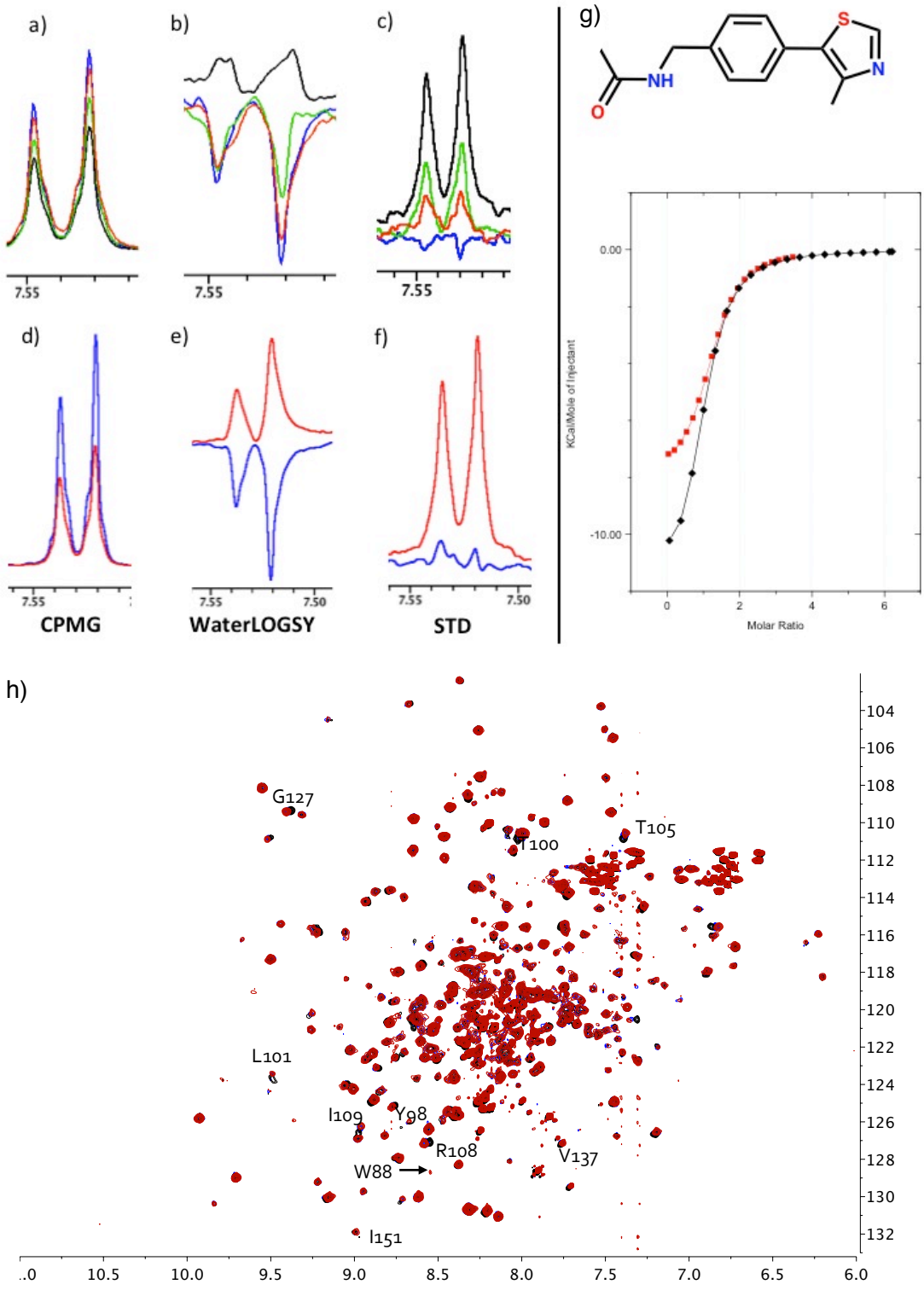


Figure S8: Binding detection using ligand-based NMR spectroscopy for **8**. Panels a), b) and c) depict spectra for VCB+**8** using set-ups 1 and 2 (red and black respectively), the compound alone (blue) and in competition with 100 μM 19-mer HIF-1α peptide under set-up 2 (green). Panels d), e) and f) depict spectra for VCB+**8** using set-up 3 (red) and compound alone (blue). g) Competitive ITC titration between **2** and **8**. Direct titration of **2** into 100 μM VCB is shown in the absence (black trace) and presence (red trace) of 3 mM of **8**. h) ^{1}H-^{15}N HSQC of 0.3 mM VCB (black contours) and when titrated with 5 mM of **8** (red contours).

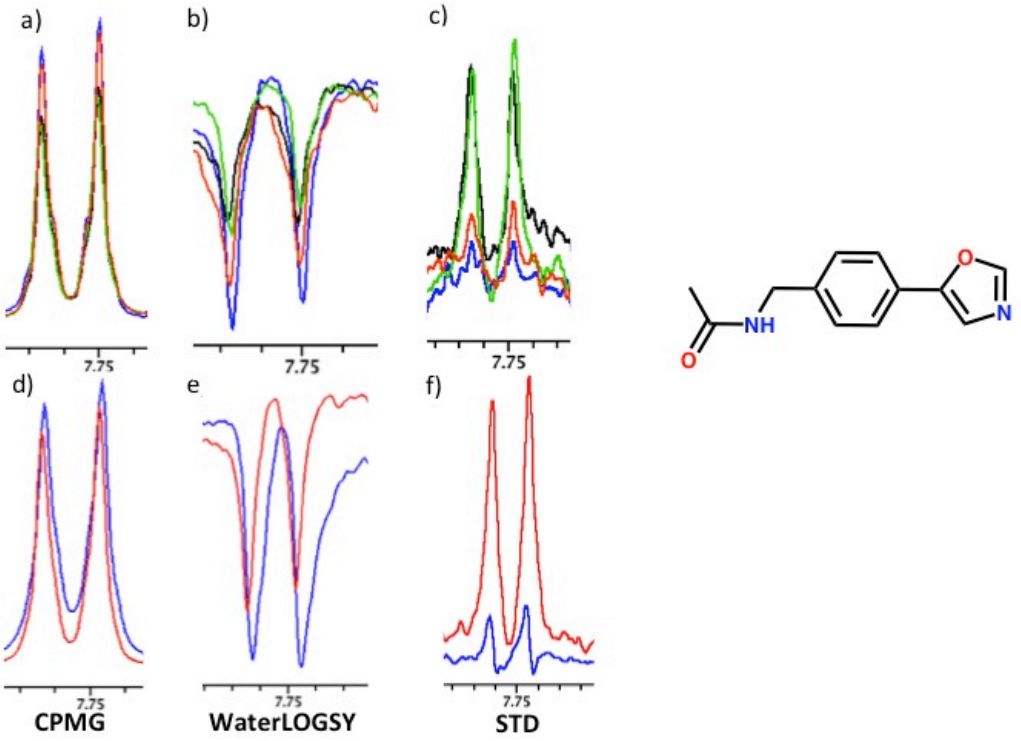


Figure S9: Binding detection using ligand-based NMR spectroscopy for **9**. Panels a), b) and c) depict spectra for VCB+**9** using set-ups 1 and 2 (red and black respectively), the compound alone (blue) and in competition with 100 μM 19-mer HIF-1α peptide under set-up 2 (green). Panels d), e) and f) depict spectra for VCB+**9** using set-up 3 (red) and compound alone (blue).

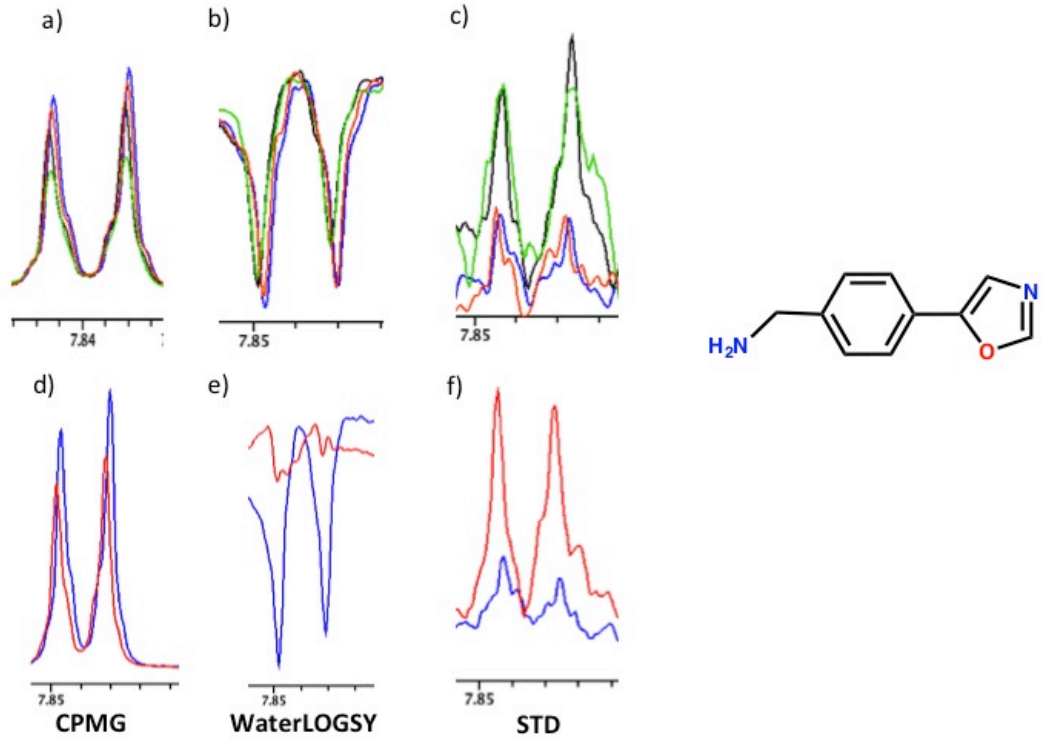


Figure S10: Binding detection using ligand-based NMR spectroscopy for **10**. Panels a), b) and c) depict spectra for VCB+**10** using set-ups 1 and 2 (red and black respectively), the compound alone (blue) and in competition with 100 μM 19-mer HIF-1α peptide under set-up 2 (green). Panels d), e) and f) depict spectra for VCB+**10** using set-up 3 (red) and compound alone (blue).

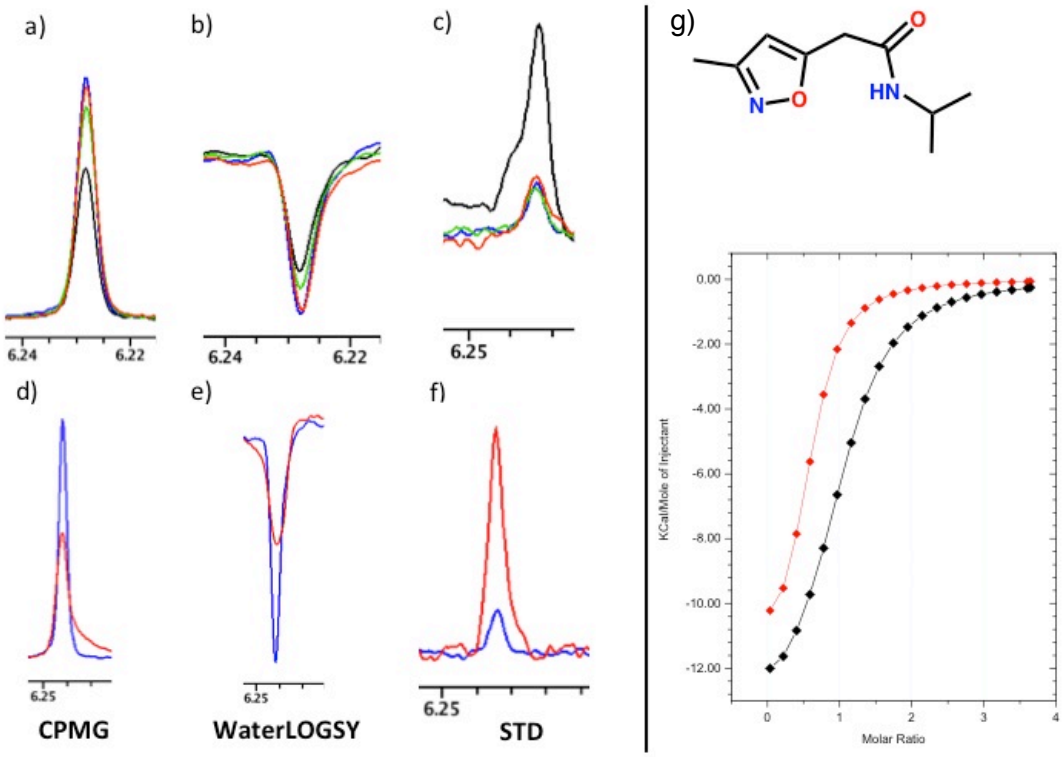


Figure S11: Binding detection using ligand-based NMR spectroscopy for **11**. Panels a), b) and c) depict spectra for VCB+**11** using set-ups 1 and 2 (red and black respectively), the compound alone (blue) and in competition with 100 μM 19-mer HIF-1α peptide under set-up 2 (green). Panels d), e) and f) depict spectra for VCB+**11** using set-up 3 (red) and compound alone (blue). g) Competitive ITC titration between **2** and **11**. Direct titration of **2** into 100 μM VCB is shown in the absence (black trace) and presence (red trace) of 3 mM of **11**.

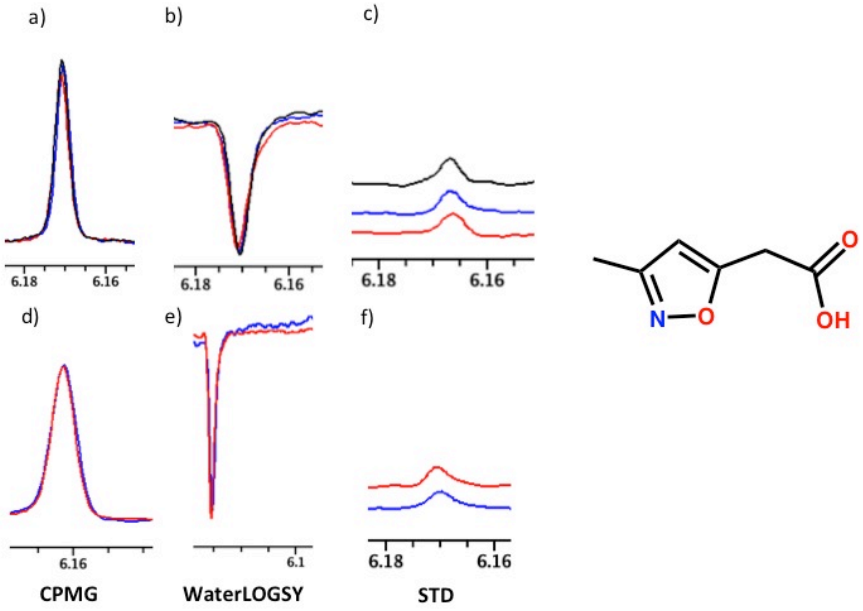


Figure S12: Binding detection using ligand-based NMR spectroscopy for **12**. Panels a), b) and c) depict spectra for VCB+**12** using set-ups 1 and 2 (red and black respectively). Panels d), e) and f) depict spectra for VCB+**12** using set-up 3 (red) and compound alone (blue)

7 – Group Epitope Mapping (GEM)

Table S2. GEM calculations for **6**.

Proton	STD amplification factor[6]	GEM (%)
a	0.91	100
b	0.91	100
c	0.72	79
d	0.20	22
e	0.36	39

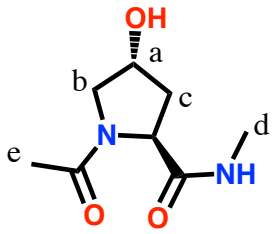


Table S3. GEM calculations for **8**.

Proton	STD amplification factor[6]	GEM (%)
a	1.42	100
b	0.81	57
c	0.73	51
d	0.35	25
e	0.53	38
f	0.49	35

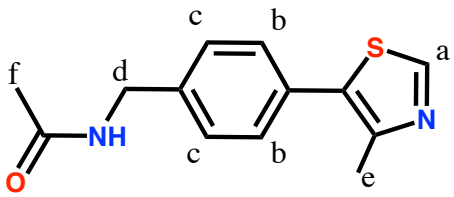
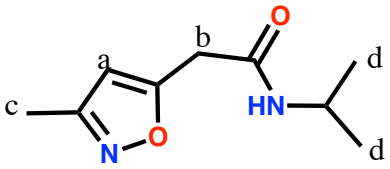


Table S4. GEM calculations for **11**.

Proton	STD amplification factor[6]	GEM (%)
a	0.81	100
b	0.18	22
c	0.19	24
d	0.43	53



8 - References

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(6) Mayer, M.; Meyer, B. *Angew. Chem. Int. Ed.* **1999**, *38*, 1784–1788.