Supplementary Note 1

Synthetic procedures and characterization for linker intermediates

General synthetic procedures

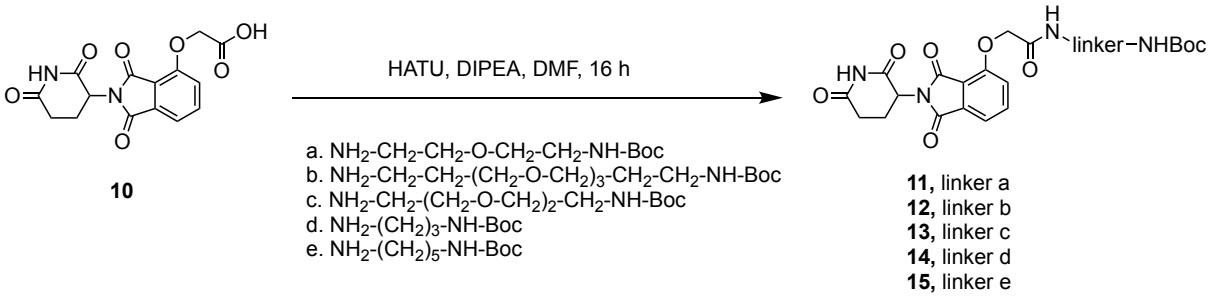
Unless otherwise stated, all reactions were performed at room temperature. Furthermore, dry solvents were used as bought from Sigma-Aldrich, Alfa Aesar and others and used without additional purification. Reactions were monitored via thin layer chromatography. Aluminium coated with Silica gel plates (particle size: 60 µm) from Merck were used. Purification of synthetic products was achieved by column chromatography using silica gel 60 (particle size: 0.04 - 0.63), bought from Merck. The Reveleris Prep system (Grace) was used for flash chromatography (FC) with Reveleris/Interchim silica and RP18 100-10 C18 (250x20 mm) cartridges, respectively.

For analyses and characterization, NMR spectrometers DPX250 and AV400 (Bruker) were used to record ^{1}H and ^{13}C spectra. For measurements, deuterated solvents $CDCl_3$ and DMSO-*d6* were used. Following abbreviations were used to describe fine structures: ‘s’ for singulett, ‘d’ for dublett, ‘t’ for triplett, ‘m’ for multiplett. Chemical shifts are displayed in ppm (parts per million). Electrospray ionisation (ESI) mass spectrometry was performed on a Thermo Fischer Surveyor MSQ c was used. High-resolution mass spectra were recorded on a MALDI LTQ ORBITRAP XL device from Thermo Fisher Scientific. For HPLC analysis an Agilent - 1260 infinity was used with a preparative Eclipse XDB-C18 5/4m (4.6 x 250 mm) column.

HPLC method for purity test (solvents included also 0.1% TFA):

Total duration	**H_2O - 98%**	→	**ACN - 98%**	→	**H_2O - 98%**
19 min -	2 min	8 min	5 min	2 min	2 min

General Method for Thalidomide-Linker coupling



10

11, linker a
12, linker b
13, linker c
14, linker d
15, linker e

2-[[2-(2,6-Dioxo-3-piperidinyl)-2,3-dihydro-1,3-dioxo-1*H*-isoindol-4-yl]oxy]aceticacid - Compound **10** (310 mg, 0.93 mmol, 1.0 eq) was dissolved in 5 mL DMF under argon, HATU (418 mg, 1.02 mmol,1.1 eq) and DIPEA (324 µL, 1.86 mmol, 2.0 eq) were added. After 15 minutes stirring at RT, solutions of the respective linkers a-e (1.12 mmol, 1.2 eq) in 2 ml DMF were added and the reaction mixtures were stirred overnight. The mixture was transferred into a separation funnel, and the reaction mixture was extracted with water (20 mL) and ethyl acetate (30 mL). The layers were separated and the aqueous layers were extracted three times with 10 mL ethyl acetate. The organic layers were combined, dried over $MgSO_4$ and concentrated. The residue was purified with FC (DCM/MeOH, gradient from 99:1 to 95:5). The products were obtained as colourless oil.

Intermediate **11**: ***tert*-butyl (2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)ethoxy)ethyl)carbamate** (Th-PEG1-NHBoc) was obtained with yield of 69 % (339 mg, 0.93 mmol).

^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.75 (m, 1H, arylCH), 7.66 (s, 1H, NH), 7.51(m, 1H, arylCH), 7.15 (m, 1H, arylCH), 4.64 (s, 2H, CH_2), 4.44 (m, 1H, CH), 3.75-3.68 (m, 4H), 3.60-3.40 (m, 2H), 2.87 (m, 2H), 2.79 (m,1H), 2.65 (m,2H), 2.44 (m, 1H), 1.39 (s,9H)

^{13}C NMR (125 MHz, CDCl3) δ 171.21, 169.75, 169.12, 168.34, 166.81, 166.14, 156.41, 137.16, 133.78, 119.70, 118.11, 117.37, 79.58, 72.93, 70.07, 69.80, 69.33, 67.70, 49.42, 42.29, 40.55, 39.91, 34.70, 31.49, 28.51, 22.90, 21.12.

ESI (+): found 563.26 [M+H$^+$]

calculated 562.58

Intermediate **12**: ***tert*-butyl (1-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)-2-oxo-7,10,13-trioxa-3-azahexadecan-16-yl)carbamate** (Th-PEG3-NHBoc) was obtained with yield of 57 % (285 mg, 0.53 mmol).

^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.86 [ppm] (s, 1H, NH), 7.73 (m, 1H, arylCH), 7.51(m, 1H, arylCH), 7.18 (m, 1H, arylCH), 4.22 (m, 2H, CH_2), 4.64 (s, 2H, CH_2), 3.68-3.40 (m, 13H), 3.18 (m, 2H, CH_2), 3.9-2.75 (m, 6H), 2.15 (m,2H), 1.86 (m, 1H), 1.76 (m, 1H), 1.42 (s,9H,Boc)

^{13}C NMR (125 MHz, CDCl3) - δ [ppm] 171.14, 168.19, 166.78, 166.64, 166.04, 154.61, 149.60, 137.05, 133.62, 119.71, 118.18, 117.43, 70.89, 70.45, 70.18, 70.12, 69.39, 68.76, 68.22, 49.32, 38.40, 36.51, 31.42, 29.62, 29.31, 28.46, 22.70, 4.92.

ESI (+): found 635.47 [$M+H^+$]

calculated 634,68

Intermediate **13**: ***tert*-butyl (2-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)ethoxy)ethoxy)ethyl)carbamate** (Th-PEG2-NHBoc) was obtained with yield of 73 % (316 mg, 0.68 mmol).

^{1}H-NMR (500 MHz, $CDCl_3$) δ [ppm] 8.77 (s, 1H, NH), 7.67(m, 1H, arylCH), 7.56 (s, 1H, NH), 7491(m, 1H, arylCH), 7.12 (m, 1H, arylCH), 4.92 (m, 1H, CH_2), 4.60 (s, 2H, CH_2), 3.65-3.48 (m, 10H) , 3.23 (m, 2H), 2.87 (m, 1H), 2.65 (m,1H), 2.11 (m, 1H), 1.36 (s, 9H, Boc)

^{13}C NMR (100 MHz, CDCl3)- δ [ppm] 171.21, 168.02, 167.03, 166.51, 165.53, 156.26, 154.36, 136.99, 133.58, 119.28, 118.10, 116.99, 114.51, 79.95, 70.64, 70.24, 70.03, 69.76, 69.55, 67.94, 49.34, 40.43, 39.20, 31.40, 28.29, 27.39, 22.64.

ESI (+): found 563.26 [$M+H^+$]

calculated 562.58

Intermediate **14**: ***tert*-butyl (3-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)propyl)carbamate** was obtained with yield of 60 % (233 mg, 0.60 mmol).

^{1}H-NMR (400 MHz, $CDCl_3$) δ [ppm] 8.48 (1H, NH), 7.68 (m, 1H, arylCH), 7.60 (m, 1H, NH), 7.48 (m, 1H, arylCH), 7.15 (m, 1H, arylCH) 4.92 (m, H, CH_2), 4.59 (m, 2H, CH_2), 4.35 (m, 2H, CH_2), 3.08 (m, 2H, CH_2), 2.28-2.68 (m, 3H), 2.07 (m, 1H), 1.68 (m, 1H), 1.36 (s, 9H, Boc)

^{13}C NMR (125 MHz, CDCl3) δ [ppm] 171.08, 168.27, 167.01, 166.12, 156.15, 154.55, 137.07, 133.24, 119.68, 118.23, 117.44, 78.90, 68.73, 68.49, 49.70, 41.31, 40.53, 38.85, 31.43, 29.70, 28.77, 28.43, 27.80, 24.05, 22.74.

ESI (+): found 489,23 [$M+H^+$]

calculated 488.50

Intermediate **15**: ***tert*-butyl (5-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)pentyl)carbamate** was obtained with a yield of 53% (206 mg, 0.50 mmol).

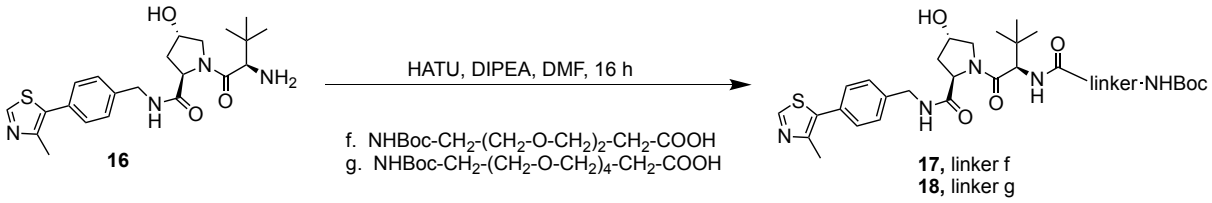
^{1}H-NMR (400 MHz, $CDCl_3$) δ [ppm] 9.00 (1H, NH), 7.66 (m, 1H, arylCH), 7.46 (m, 2H,arylCH, NH), 7.12 (m, 1H, arylCH), 4.93 (m, H, CH_2), 4.53 (m, 2H, CH_2), 3.40 (s, 1H, NH), 3.25 (m, 1H, CH_2), 3.06 (m, 2H, CH_2), 2.84-2.60 (m, 3H), 2.10 (m, 1H), 1.72 (s, 1H, NH), 1.55-1.47 (m, 4H), 1.36 (s, 9H, Boc)

^{13}C NMR (125 MHz, CDCl3) δ [ppm] 171.24, 168.27, 166.66, 166.12, 155.81, 154.55, 136.78, 133.72, 119.34, 118.08, 117.07, 68.13, 49.32, 40.21, 39.01, 31.43, 29.70, 28.77, 27.86, 24.05, 23.18.

ESI (+): found 517.29 [M+H+]

calculated 516,55

General Method for VHL-Linker coupling



16

17, linker f
18, linker g

Compound **16**, VHL ligand (92 mg, 0.214 mmol, 1.0 eq) was dissolved in 6 mL DMF (reaction mixture (A)) under argon. A mixture of the respective linker f-g (0.236 mmol, 1.1 eq) and HATU (92 mg, 0.241 mmol, 1.1 eq) were dissolved in 6 mL DMF in a separate flask (reaction mixture (B)) under argon atmosphere. Both flasks were cooled to 0° C. DIPEA (117 µL, 0.65 mmol, 4.0 eq) was added to flask(A), 0.12 ml (117 µL, 0.65 mmol, 4 eq) to flask(B). The ice bath was removed and both solutions were stirred for 20 min, then flask(B) was transferred into flask(A) and reaction mixtures was stirred overnight. The orange mixture was transferred into a separation funnel and brine was added. The layers were separated and the aqueous layer was extracted 5 times with DCM The organic layers were combined, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The remaining residue was purified with FC (DCM/MeOH, 100:0-95:5). The intermediate was obtained as colourless oil.

Intermediate **17:** ***tert*-butyl (2-(2-(3-*(((S)-1-((2S,4R)*-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-3-oxopropoxy)ethoxy)ethyl)carbamate** (VHL-PEG2-NHBoc) was obtained with a yield of 59 % (75 mg, 0.13 mmol).

^{1}H-NMR: (250 MHz, $CDCl_3$) δ [ppm] 8.67 (s, 1H, thiazoleCH), 7.33 (s, 4H,arylCH), 4.81 - 4.42 (m, 4H), 4.29 (d, J = 15.4 Hz, 1H), 4.04 (d, J = 11.3 Hz, 1H), 3.76 - 3.39 (m, 9H), 3.22 (s, 2H), 2.86 (t, J = 19.3 Hz, 4H), 2.57 - 2.35 (m, 6H), 1.40 (s, 9H, Boc), 0.97 (d, J = 17.4 Hz, 9H, *tert*-Butyl)

ESI (+): found 712.51 [M + Na^+],

656.44 [M -*tert*-Butyl+Na^+],

calculated 689.86

Intermediate **18:** ***tert*-butyl ((S)-16-(*(2S,4R)*-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidine-1-carbonyl)-17,17-dimethyl-14-oxo-3,5,8,11-**

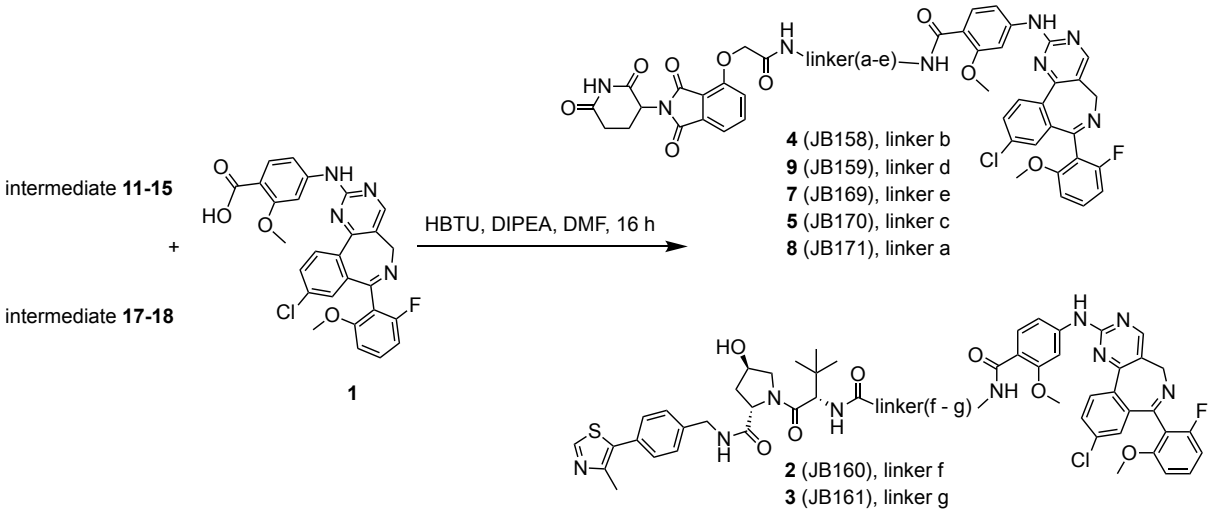
tetraoxa-15-azaoctadecyl)carbamate (VHL-PEG4-NH_2) was obtained with yield of 52 % (75 mg, 0.11 mmol).

^{1}H-NMR: (250 MHz, $CDCl_3$) δ [ppm] 8.67 (s, 1H,thiazoleCH), 7.34 (s, 4H, arylCH), 4.70 (t, J = 8.0 Hz, 1H), 4.58 - 4.43 (m, 3H), 4.32 (dd, J = 15.0, 5.3 Hz, 1H), 4.07 (d, J = 11.4 Hz, 1H), 3.92 (s, 1H), 3.76 - 3.44 (m, 18H), 3.26 (t, J = 10.3 Hz, 2H), 2.58 - 2.38 (m, 7H), 2.12 (dd, J = 13.3, 8.2 Hz, 1H), 1.47 (t, J = 7.2 Hz, 1H), 1.41 (d, J = 4.4 Hz, 9H, Boc), 0.93 (s, 9H, *tert*-Butyl).

ESI (+): found	800.59 [M +Na^+],
	744.48 [M -*tert*-Butyl+Na^+]
calculated	677.85

Synthetic procedures and characterization for PROTACs

General Method for Coupling of Alisertib with VHL and Thalidomide derivates



The intermediates were dissolved in DCM/TFA (20 vol%) and stirred at room temperature for 2 h. After complete deprotection, the reaction mixture was concentrated under reduced pressure. The deprotected intermediate compounds **11-15** (thalidomide) and **17-18** (VHL), 0.02 mmol (1.0 eq) were dissolved in 1.5 mL DMF. HBTU (7.6 mg, 0.02 mmol, 1.0 eq) was added. After dissolving, compound **1** Alisertib

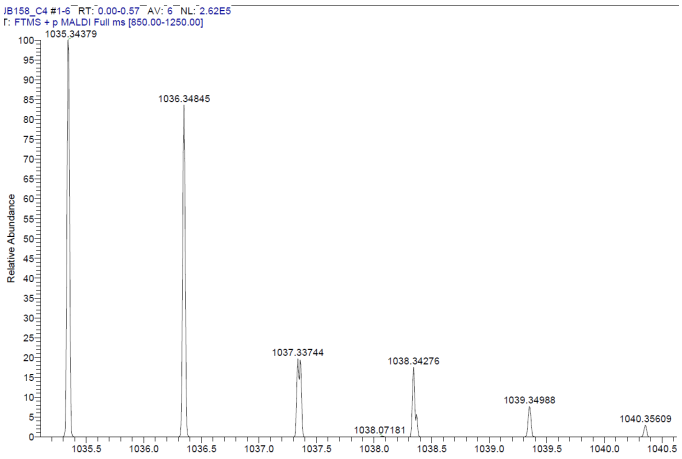
(10 mg, 0.02 mmol, 1.0 eq) was added, followed by DIPEA (14 µL, 0.08 mmol, 4.0 eq). pH was adjusted to 8-10 by adding more equivalents DIPEA. Reaction mixtures were stirred overnight. The solvent was removed using a SpeedVac concentrator. The residues were purified and characterised via preparative and analytical HPLC. All reactions were done with 10 mg Alisertib except JB170. This compound was synthesized first with 5 mg and 50 mg Alisertib.

Compound **4:** **4-((9-chloro-7-(2-fluoro-6-methoxyphenyl)-*5H*-benzo[c]pyrimido [4,5-e]azepin-2-yl)amino)-*N*-(1-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)-2-oxo-7,10,13-trioxa-3-azahexadecan-16-yl)-2-methoxybenzamide**
Thalidomide-PEG3-Alisertib (JB158) was obtained as white solid with a yield of 90% (9 mg, 0.009 mmol).

HPLC (t_R) = 12.72 min

HPLC purity: (λ= 254.4 nm) 97.0 %

HRMS-ESI (m/z) for $[M+H]^+$ - calculated: 1035.34500; found: 1035,34379



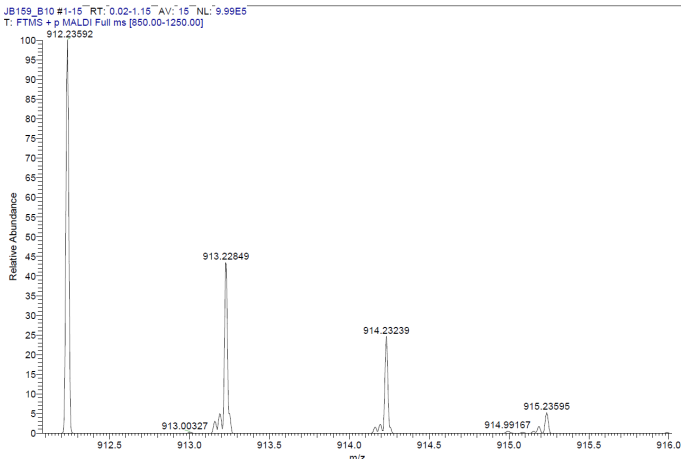
^{1}H-NMR: (400 MHz, DMSO-*d6*) δ 11.00 (s, 1H, NH), 10.04 (s, 1H, NH), 8.61 (m, 1H, arylCH), 8.77 (m, 1H, arylCH), 8.20 (1H, arylH), 8.17 (t, J=8.0 Hz, 1H, arylCH), 8.00 (t, J=8.0 Hz, 1H, arylCH), 7.96 (s, 1H, NH), 7.80 (m, 3H, arylH), 7.42 (m, 4H, arylH), 7.21 (s, 1H, NH), 5.11 (m, 1H), 4.77 (s, 2H, CH_2), 3.92 (s, 3H, OCH_3), 3.57 (s, 3H, OCH_3), 3.64-3.75 (m, 10H), 3.51-3.34 (m, 10H), 3.13 (m, 1H), 2.89 (m, 1H), 2.60 (m, 1H), 2.07 (m, 1H)

Compound **9:** **4-((9-chloro-7-(2-fluoro-6-methoxyphenyl)-*5H*-benzo[c]pyrimido [4,5-e]azepin-2-yl)amino)-*N*-(6-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)hexyl)-2-methoxybenzamide** Thalidomide-$(CH_2)_3$-Alisertib (JB159) was obtained as white solid with a yield of 70% (6 mg, 0.007 mmol).

HPLC (t_R) = 12.52

HPLC purity: (λ= 254.4 nm) 99.1 %

HRMS-ESI (m/z) for $[M+K]^+$ - calculated: 912.23208; found: 912.23592;



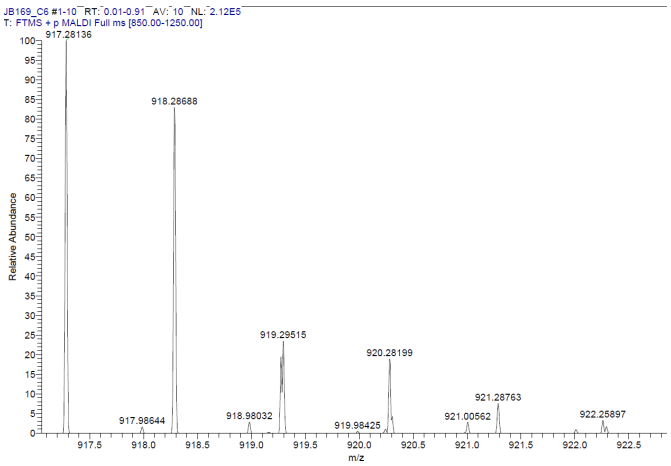
^{1}H-NMR: (400 MHz, DMSO-*d6*) δ [ppm] 10.80 (s, 1H, NH), 10.17 (s, 1H, NH), 8.71 (m, 1H, arylCH), 8.56 (m, 1H, arylCH), 8.20 (d, J=8.3 Hz, 1H), 8.15 (m, 1H, arylCH), 7.99 (m, 1H, arylCH), 7.96 (s, 1H, NH), 7.80 (m, 3H, arylH), 7.42 (m, 4H, arylH), 7.23 (s, 1H, NH), 5.21 (m, 1H), 4.65 (s, 2H, CH_2), 3.88 (s, 3H, OCH_3), 3.56 (s, 3H, OCH_3), 3.57-3.65 (m, 4H), 3.51-3.34 (m, 7H), 3.13 (m, 1H), 2.89 (m, 1H), 2.59 (m, 1H), 2.06 (m, 1H)

Compound **7:** **4-((9-chloro-7-(2-fluoro-6-methoxyphenyl)-*5H*-benzo[c]pyrimido [4,5-e]azepin-2-yl)amino)-*N*-(5-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)pentyl)-2-methoxybenzamide** Thalidomide-$(CH_2)_5$-Alisertib (JB169) was obtained as white solid with a yield of 61% (5mg, 0.006 mmol).

HPLC (t_R) =13.01

HPLC purity: (λ= 254.4 nm) 98.5 %

HRMS-ESI (m/z) for $[M+H]^+$ - calculated: 917.28201; found: 917.28136;



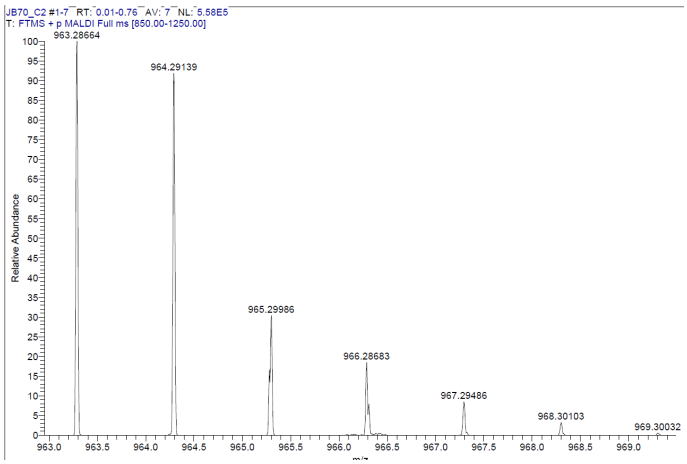
^{1}H NMR (500 MHz, Chloroform) δ [ppm] 8.64 (s, 1H, NH), 8.33 (s, 1H, arylCH), 7.99 (s, 1H, arylCH), 7.88 (s, 1H, NH), 7.74 (s, 1H, arylCH), 7.50 (m, 3H), 7.20 (m, 2H), 7.05 (s, 1H), 6.76 (s, 1H), 6.65 (m, 2H), 6.53 (s, 1H), 5.66 (s, 1H), 5.03 – 4.86 (m, 2H), 4.87 – 4.73 (m, 2H), 4.44 (s, 1H), 3.99 (s, 1H), 3.89 – 3.76 (m, 3H), 3.77 – 3.65 (m, 3H), 3.26 (dd, J = 15.5, 10.4 Hz, 4H), 2.57 (t, J = 35.1 Hz, 3H), 2.17 (s, 1H), 1.76 – 1.51 (m, 4H), 1.44 – 1.00 (m, 2H).

Compound **5**: **4-((9-chloro-7-(2-fluoro-6-methoxyphenyl)-*5H*-benzo[c]pyrimido [4,5-e]azepin-2-yl)amino)-*N*-(2-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)ethoxy)ethoxy)ethyl)-2-methoxybenzamide**
Thalidomide-PEG2-Alisertib (JB170) was obtained as white solid with a yield of 48% (5 mg, 0.005 mmol).

HPLC (t_R) =12.54

HPLC purity: (λ= 254.4 nm) 99.3 %

HRMS-ESI (m/z) for $[M+H]^+$ - calculated: 963.28749; found: 963.28664;



^{1}H-NMR: (400 MHz, DMSO-*d6*) δ [ppm] 11.11 (s, 1H, NH), 10.17 (s, 1H, NH), 8.71 (m, 1H, arylCH), 8.56 (m, 1H, arylCH), 8.30 (d, J=8.3 Hz, 1H), 8.15 (t, J=8.1 Hz, 1H, arylCH), 8.00 (t, J=8.0 Hz, 1H, arylCH), 7.96 (s, 1H, NH), 7.80 (m, 3H, arylH), 7.42 (m, 4H, arylH), 7.23 (s, 1H, NH), 5.11 (m, 1H), 4.77 (s, 2H, CH_2), 3.92 (s, 3H, OCH_3), 3.57 (s, 3H, OCH_3), 3.54-3.45 (m, 6H), 3.51-3.34 (m, 8H), 3.13 (m, 1H), 2.89 (m, 1H), 2.59 (m, 1H), 2.06 (m, 1H)

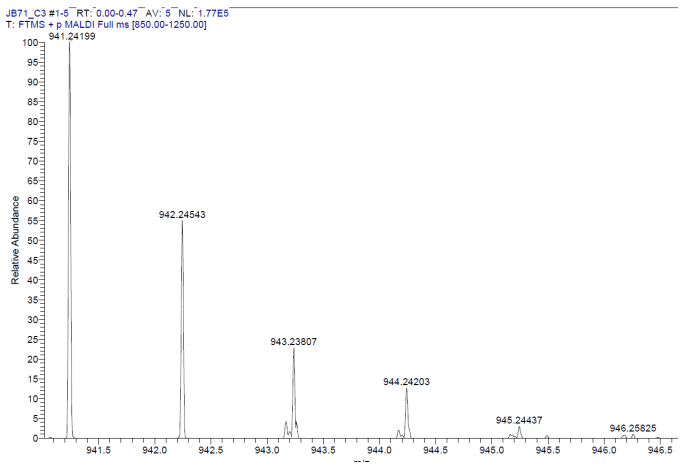
Compound **8**: **4-((9-chloro-7-(2-fluoro-6-methoxyphenyl)-*5H*-benzo[c]pyrimido [4,5-e]azepin-2-yl)amino)-*N*-(*2*-(*2*-(*2*-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)ethoxy)ethyl)-2-methoxybenzamide**

Thalidomide-PEG1-Alisertib (JB171) was obtained as white solid with a yield of 75% (7 mg 0,007 mmol).

HPLC (t_R) = 12.65

HPLC purity: (λ= 254.4 nm) 95.9 %

HRMS-ESI (m/z) for $[M+Na]^+$ - calculated: 941.24322; found: 941.24199;



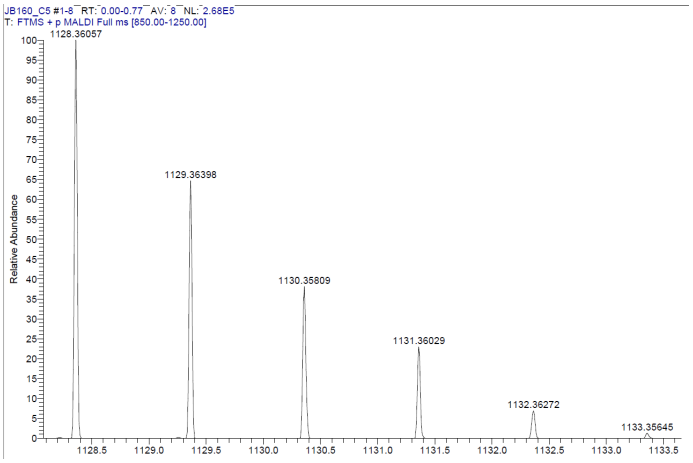
^{1}H-NMR: (400 MHz, DMSO-*d6*) δ [ppm] 10.80 (s, 1H, NH), 10.02 (s, 1H, NH), 8.61 (m, 1H, arylCH), 8.66 (m, 1H, arylCH), 8.20 (d, J=8.3 Hz, 1H), 8.18 (t, J=8.1 Hz, 1H, arylCH), 8.03 (t, J=8.0 Hz, 1H, arylCH), 7.78 (s, 1H, NH), 7.83 (m, 3H, arylH), 7.32 (m, 4H, arylH), 7.23 (s, 1H, NH), 5.11 (m, 1H), 4.79 (s, 2H, CH_2), 3.99 (s, 3H, OCH_3), 3.68 (s, 3H, OCH_3), 3.58-3.53 (m, 4H), 3.50-3.33 (m, 6H), 3.12 (m, 1H), 2.89 (m, 1H), 2.57 (m, 1H), 2.06 (m, 1H)

Compound **2:** ***(2S,4R)*-1-((S)-13-(*tert*-butyl)-1-(4-((9-chloro-7-(2-fluoro-6-methoxyphenyl)-*5H*-benzo[c]pyrimido[4,5-e]azepin-2-yl)amino)-2-methoxyphenyl)-1,11-dioxo-5,8-dioxa-2,12-diazatetradecan-14-oyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide** VHL-PEG2-Alisertib (JB160) was obtained as white solid with a yield of 43% (4.5 mg, 0.004 mmol).

HPLC (t_R) =12.62

HPLC purity: (λ= 254.4 nm) 98.2 %

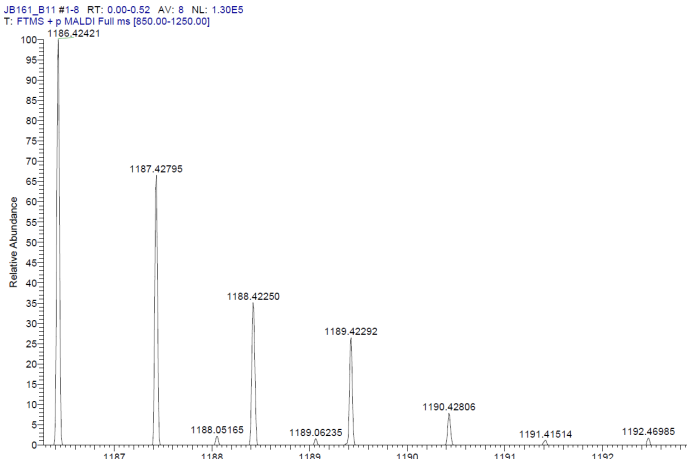
HRMS-ESI (m/z) for $[M+K]^+$ - calculated: 1128.36171; found: 1128.36057;



Compound **3:** ***(2S,4R)*-1-((S)-18-(*tert*-butyl)-1-(4-((9-chloro-7-(2-fluoro-6-methoxyphenyl)-*5H*-benzo[c]pyrimido[4,5-e]azepin-2-yl)amino)-2-methoxyphenyl)-1,16-dioxo-5,7,10,13-tetraoxa-2,17-diazanonadecan-19-oyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide** VHL-PEG4-Alisertib (JB161) was obtained as white solid with a yield of 62% (7 mg, 0.006 mmol).

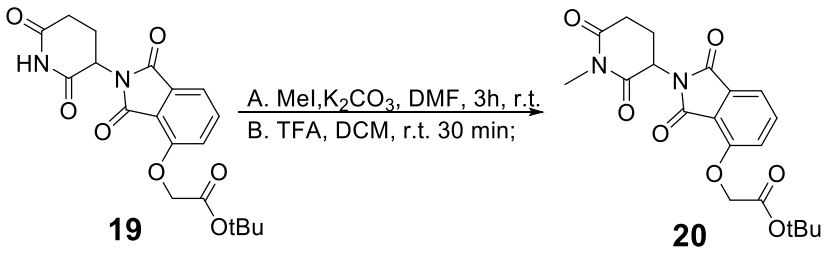
HPLC (t_R) =12.66

HRMS-ESI (m/z) for $[M+Na]^+$ - calculated: 1186.42455; found: 1186.42421;



Synthesis of negative control (JB211)

Method for methylation of glutarimide-ring of thalidomide



Compound **19**: ***tert*-butyl 2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl) oxy)acetate** (100 mg, 0.26 mmol, 1.0 eq) and methyl iodide (36 mg, 0.26 mmol, 1.0) were dissolved in 1 mL DMF under Ar. (54 mg, 0.39 mmol, 1.5 eq) K_2CO_3 was added. Reaction mixture was stirred 3 h at RT.

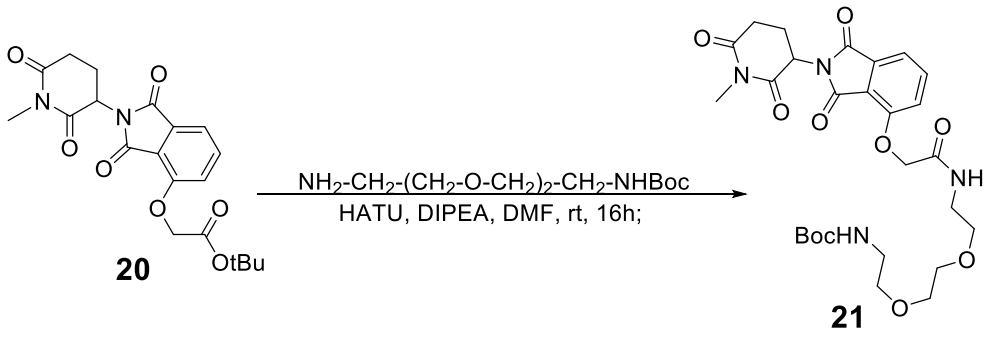
Ethyl acetate (1 mL) and water (1 mL) were added. The layers were separated, the organic layer was dried over $MgSO_4$ and concentrated. The crude product (intermediate **20**) was used without purification. Yield: 61% (63 mg, 0.16 mmol)

Intermediate **20**: ***tert*-butyl 2-((2-(2,6-dioxo-N-methylpiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetate**

^{1}H NMR: (400 MHz, $CDCl_3$, 300 K): δ [ppm] 7.94 (s, 1H), 7.67 (dd, J = 8.4, 7.3 Hz, 1H), 7.52 (d, J= 6.8 Hz, 1H), 7.11 (d, J = 8.3 Hz, 1H), 4.97 (dd, J = 12.3, 5.3 Hz, 1H), 4.79 (s, 2H), 2.95 - 2.89 (m, 1H), 2.85 - 2.71 (m, 2H), 2.28 (s, 3H, CH_3), 2.14 (m, 1H), 1.48 (s, 9H)

ESI (+):	found	411.03 [M+Na]
	calculated	388.03

*Method for intermediate **20** -Linker c coupling*



To remove the *tert*-Butyl protection group, intermediate **20 (***tert*-butyl 2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetate) (60 mg, 0.15 mmol, 1.0 eq.) was dissolved in 1 mL DCM and trifluoroacetic acid (40 Vol.%) was added. After 30 min the solvent was removed under reduced pressure. Deprotected product was again dissolved in 2 mL DMF under argon, HATU (57 mg, 0.15 mmol, 1.0 eq) and DIPEA (102 µl, 0.60 mmol, 4.0 eq) were added. After 15 minutes a solution of linker c (44 mg, 0.18 mmol, 1.2 eq) in 2 mL DMF was added and reaction mixtures was stirred overnight. The mixture was transferred into a separation funnel, water (5 mL) and ethyl acetate (10 mL) were added. The layers were separated, and the aqueous layer was extracted 3 times with ethyl acetate. The organic layers were combined, dried over $MgSO_4$ and concentrated. The crude product (intermediate **21**) was used without purification. Yield: 31% (27 mg, 0.05 mmol)

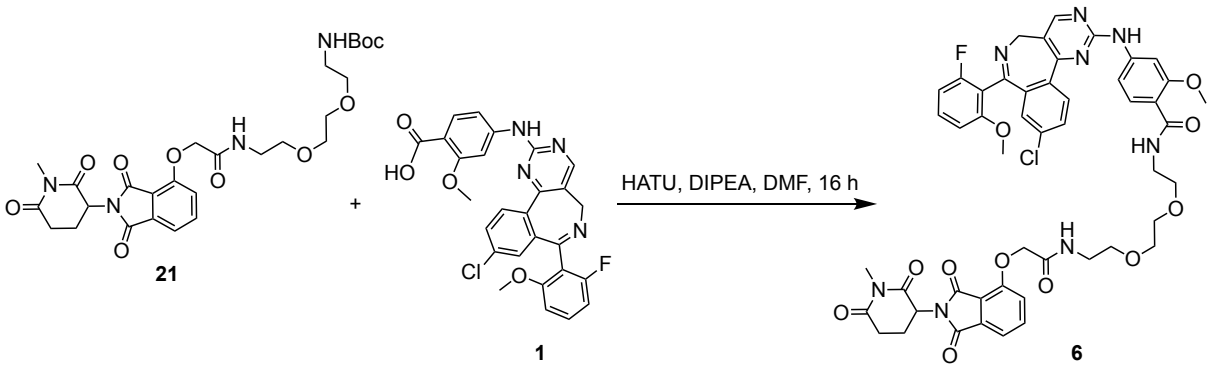
Intermediate **21:** ***tert*-butyl (2-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)ethoxy)ethoxy)ethyl)carbamate**

^{1}H NMR: (250 MHz, $CDCl_3$): δ [ppm] 7.67 (m, 1H),7.46 (s, 1H) 7.32 (d, J= 6.8 Hz, 1H), 4.97 (m, 2H), 4.79 (s, 2H), 3.62-3.40 (m, 10H), 3.20 (m, 3H), 2.95 - 2.89 (m, 1H), 2.85 - 2.71 (m, 2H), 2.28 (s,3H, CH_3), 2.14 (m, 1H), 1.48 (s, 9H).

ESI (+): found 576.60 [M+Na$^+$]

calculated 576.60

*Coupling of Alisertib with intermediate **21***

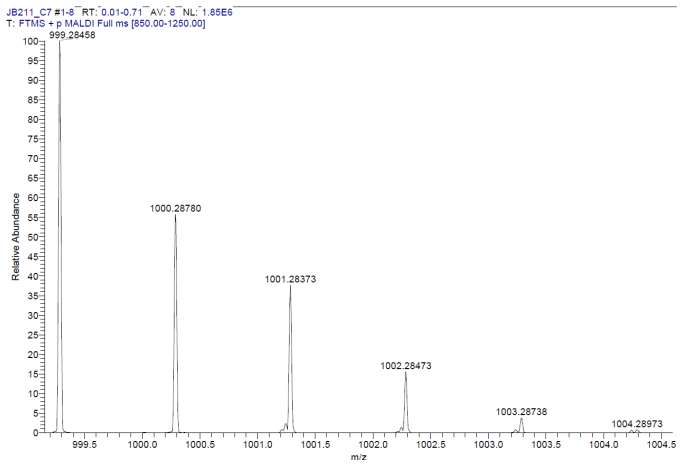


To remove the *tert*-Butyl protection group, intermediate **21** (23 mg, 0.04 mmol) was dissolved in 1 mL DCM and trifluoroacetic acid (40 Vol.%) was added. The final compound was synthesized as described in *general method for Coupling of Alisertib with Thalidomide derivates*. The product **6** (JB211) was obtained as white powder 89% (35 mg, 0.036 mmol).

Compound **6**: **4-((9-chloro-7-(2-fluoro-6-methoxyphenyl)-*5H*-benzo[c]pyrimido [4,5-e]azepin-2-yl)amino)-2-methoxy-*N*-*(2*-*(2*-*(2*-*(2*-((2-(1-methyl-2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)ethoxy)ethoxy) ethyl)benzamide** (JB211)

^{1}H NMR: (600 MHz, $CDCl_3$) δ [ppm] 10.18 (s, 1H), 8.72 (s, 1H), 8.30 (d, J = 8.4 Hz, 2H), 8.16 (t, J = 5.4 Hz, 2H), 8.01 - 7.97 (m, 3H), 7.86 - 7.78 (m, 3H), 7.48 - 7.40 (m, 3H), 6.88 (s, 1H), 5.48 (s, 1H), 5.03 - 4.85 (m, 2H), 4.88 - 4.73 (m, 2H), 4.44 (s, 1H), 3.91 - 3.70 (m, 11H), 3.26 - 3.11 (m, 3H), 3.06 (s, 1H), 2.58 (m, 2H), 2.44 (s, 1H).

HRMS-ESI (m/z) for $[M+H]^+$ - calculated: 999.28508; found: 999.28458;


JB211_C7 #1-8 RT: 0.01-0.71 AV: 8 NL: 1.85E6
T: FTMS + p MALDI Full ms [850.00-1250.00]
999.28458
1000.28780
1001.28373
1002.28473
1003.28738
1004.28973
Relative Abundance
m/z

Supplementary Note 2

Structure preparation

Preparation of the complex of Aurora-A with MLN8237 (= Alisertib) started with the crystal structure of Aurora-A in complex with MLN8054 (PDB: 2X81, resolution: 2.91 Å)[1]. The missing kinase activation loop was inserted from Aurora-A PDB structure 6R4A (resolution: 1.94 Å)[2] with the help of MOE[3] by aligning the two structures based on their C_α-trace (to an RMSD of 0.925 Å), grafting the peptide chain Asp274-Gly291 from structure 6R4A into the 2X81 structure and optimizing the transferred loop along with the adjacent amino acids by energy minimization (Forcefield: Amber14:EHT, gradient: 0.001 kcal/(mol*Å)). The obtained structure was further setup with the MOE structure preparation function, to add all missing side chains and hydrogen atoms at pH=7 (Protonate3D[4]). The complex with MLN8237 was generated by docking, as described below.

The Cereblon/Lenalidomide complex was obtained from the crystal structure of Cereblon in complex with Lenalidomide and DDB1 (PDB: 4TZ4, resolution: 3.01 Å)[5]. DDB1 was removed and the remaining complex was setup in the same way as Aurora-A.

Analogously, Aurora-B was prepared after removing INCENP and VX-680 from the crystal structure of the corresponding Aurora-B complex (PDB: 4AF3, Resolution: 2.75 Å)[6]. The obtained protein structure was aligned with the Aurora-A/MLN8237 complex and the ligand atoms were transferred to Aurora-B. After pre-minimization of the entire system (protein, solvent molecules and MLN8237;Forcefield: Amber14:EHT, gradient: 1 kcal/(mol*Å)), the water molecules were removed and the ligand was further minimized in the binding pocket of Aurora-B (Forcefield: MMFF94X, gradient: 0.0001 kcal/(mol*Å)).

Small molecule docking

Docking of MLN8054 and MLN8237 was performed with GOLD v5.7.1[7] using the implemented scoring functions ASP and ChemPLP. For each ligand, 50 independent docking runs were carried out with each scoring function. The search was restricted to the binding-site region of Aurora-A defined by a 12 Å radius around atom CD2 of Leu263. The search efficiency was set to 200%. Results were rescored with DrugscoreX v0.9[8] (DSX) using CSD potentials and clustered by fconv v1.24[9] with an

RMSD-cutoff of 1.0 Å. The Aurora-A inhibitors MLN8054 and MLN8237 were built and prepared in MOE (protonation state at pH=7; energy minimization with MMFF94X, gradient: 0.0001 kcal/(mol*Å). Redocking of MLN8054 was successful with an RMSD of the top ranked pose of 0.46 Å with respect to the crystal structure. The Aurora-A/MLN8237 complex was obtained with a substructure RMSD of the top ranked pose of 0.57 Å compared to the crystal structure of MLN8054.

Using GOLD v5.7.1 with the ChemPLP scoring function, PROTACs JB158, JB159, JB169, JB170, JB171 and JB211 were docked into the two Aurora-A/Cereblon complexes obtained by protein/protein docking (described below). In both cases, the binding-site region was defined by a 17 Å radius around atom OH of Tyr87 of Aurora-A. To increase the default search length, the number of docking operations was increased manually to 1,000,000, and 100 independent docking runs were conducted for each ligand. Due to the high flexibility of the molecules, the heavy atoms of Thalidomide (with the linking oxygen atom) and the N-phenylpyrimidine-2-amine moiety of MLN8237 were used as scaffold constraints (constraint weight: 0.01). Results were rescored with DSX v0.9 using CSD potentials and including torsion potentials with a weight of 1.0. The fconv utility was used for clustering (with an RMSD-cutoff of 2.0 Å) and for calculating RMSD values with respect to the scaffolds used for docking.

Protein/protein docking

The Aurora-A/MLN8237 complex and the Cereblon/Lenalidomide complex (prepared as described above) were used for protein/protein docking within MOE (2019.0102). Settings were kept at their defaults (Pharmacophore: None; Electron Density: None; Hydrophobic Patch Potential: Off; Antibody specific options: Off; Refinement: Rigid Body (Forcefield: Amber14:EHT, gradient: 0.01 kcal/(mol*Å), Iterations: 500); Side Chains Repack From Library: On; Potential Setup Use Reaction Field: On; Final Energy Use GB/VI: On).

The Cereblon/Lenalidomide complex was defined as the “receptor”, with the atoms of Lenalidomide defining the binding site; the Aurora-A/MLN8237 complex was used as “ligand”. The 100 generated solutions were checked for compatibility with the linker used in JB170. The results on rank 1 and rank 15 were the two best-ranked solutions suitable for modification and joining the two small molecules with the corresponding linker to JB170 without major displacement after local energy minimization. Lower

ranked solutions with orientations potentially compatible with JB170 binding were not further considered here. Rescoring the docking solutions using MOE's "Protein Contacts" tool gave a GBVI-score of -10.72 kcal/mol for rank 1 and -9.21 kcal/mol for rank 15.

The Aurora-B/MLN8237 complex was docked to Cereblon/Lenalidomide in the same way as Aurora-A. The best-scored docking solution able to accommodate JB170 was found at rank 39. Rescoring with MOE's "Protein Contacts" tool resulted in an GBVI-score of -8.80 kcal/mol.

Molecular dynamics (MD) simulations

Topology and parameter files were built using the tleap module of AMBERTools18[10]. The complexes were minimized for 2,000 steps with an implicit water model (generalized Born implicit solvent model[11]) using the pmemd.MPI module of AMBERTools18. After adding sodium ions for neutralization, the systems were solvated in a box of TIP3P water[12] with a minimum protein-to-box distance of 10 Å. The simulations were performed using NAMD 2.13[13] with AMBER ff14sb[14] forcefield parameters. Ligand charges were calculated via the RESP approach based on the electrostatic potential obtained with Gaussian09[15] at the HF/6-31G* level. Periodic boundary conditions were applied, and electrostatic interactions were handled with the particle mesh Ewald methodology[16]. System equilibration was carried out after 10,000 steps of minimization, by heating from 100 K to 300 K over 500 ps at constant volume, whereby harmonic constraints (0.5 kcal/(mol*Å²)) were applied to non-solvent atoms for the first 100 ps and gradually lowered during the remaining heating process. Afterwards the systems were allowed to move freely for another 500 ps. Starting from the equilibrated systems, production runs were performed for 100 ns each, at constant pressure (1.01325 bar, Nosé-Hoover Langevin piston pressure control) and constant temperature (300 K, Langevin dynamics). 2 fs timesteps were used for integration, and coordinates for output trajectories were saved every 500 steps (1 ps). The Aurora-A/MLN8237 and Cereblon/Lenalidomide complexes were simulated for 100 ns each, to confirm their stability under dynamic conditions. For each protein/protein complex with modelled JB170, triplicates were produced resulting in 300 ns sampling per model.

Structural analysis of the trajectories was performed using cpptraj[17]. Energetic analysis using MMPBSA.py[18] was performed on the last 5 ns of a trajectory. A step-size of 100

was chosen resulting in 51 frames for calculation. OBC II was used as generalized Born model. Aurora-A and JB170 served as “receptor” and Cereblon as “ligand” during the calculations, which enabled use of the energy decomposition function to determine contributions of Aurora-A side chains to the formation of the ternary complex.

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