Supporting Information

Targeting the Allosteric Site of Oncoprotein BCR-ABL as an Alternative Strategy for Effective Target Protein Degradation

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Chemistry section

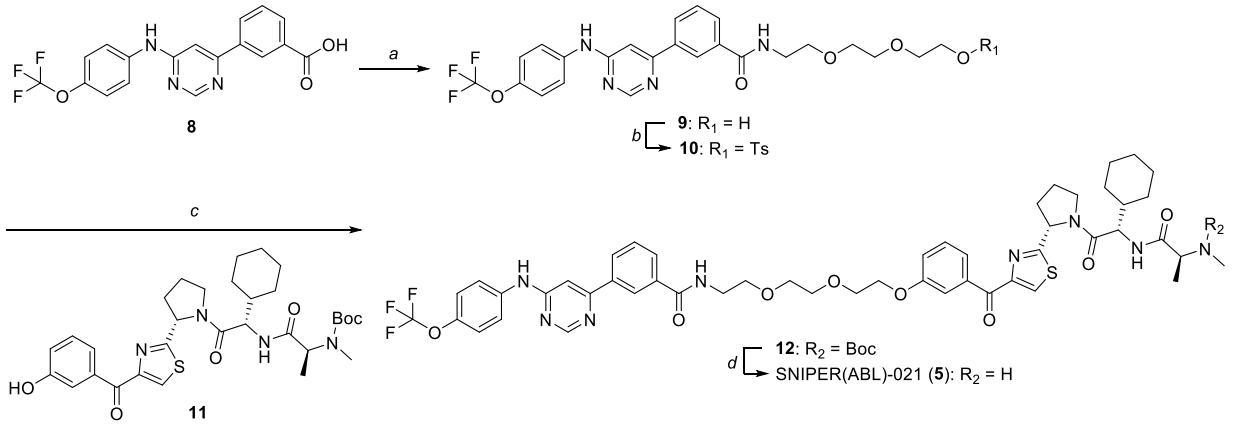
1. General remarks

The ^{1}H nuclear magnetic resonance (^{1}H NMR) spectra were acquired on a Bruker AVANCE II (300 or 400 MHz) or Bruker AVANCE+ II 600 (600 MHz) spectrometer. Chemical shifts were reported in parts per million (ppm) downfield from tetramethylsilane (δ) as the internal standard in deuterated solvent and coupling constants (J) are given in hertz (Hz). The following abbreviations are used for spin multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, dd = doublet of doublets, dt = doublet of triplets, qd = quartet of doublets, dquin = doublet of quintets, m = multiplet, and br s = broad singlet. Reaction progress was determined by thin layer chromatography (TLC) analysis on silica gel 60 F_{254} plates (Merck) or NH TLC plates (Fuji Silysia Chemical Ltd.). Column chromatography was performed with a silica gel column [(Merck Kieselgel 60, 70–230 mesh, Merck) or (Chromatorex® NH-DM1020, 100–200 mesh, Fuji Silysia Chemical Ltd.)], or with prepacked Purif-Pack columns [silica gel or NH (3-aminopropyl-functionalized) silica gel, particle size: 60 μm , Fuji Silysia Chemical Ltd.]. Low-resolution mass spectra (MS) were acquired using an Agilent LC/MS system (Agilent1200SL/Agilent6130MS or Agilent1200SL/Agilent1956MS or Agilent1200SL/Agilent6110MS), Shimadzu UFLC/MS (Shimazu LC-20AD/LCMS-2020) operating in the electron spray ionization mode (ESI+). The column used was an L-column 2 ODS (3.0 × 50 mm I.D., 3 μm, CERI, Japan) at a temperature of 40 °C and a flow rate of 1.2 or 1.5 mL/min or an Waters X-Bridge C18 (4.6 × 50 mm I.D., 3.5 μm) at a temperature of 40 °C and a flow rate of 2.0 mL/min. Mobile Phase: Condition 1: Mobile phases A and B under an acidic condition were 0.05% TFA in water and 0.05% TFA in MeCN, respectively. The ratio of the mobile phase B was increased linearly from 5% to 90% over 0.9 min and 90% over the next 1.1 min, or the ratio of the mobile phase B was increased linearly from 5% to 100% over 1.6 min and 100% over the next 1.4 min, or the ratio of the mobile phase B was increased linearly from 5% to 100% over 3.0 min and 100% over the next 1.0 min. Condition 2: Mobile phases A and B under a neutral condition were a mixture of 5 mmol/L $AcONH_4$ and MeCN (9:1, v/v) and a mixture of 5 mmol/L $AcONH_4$ and MeCN (1:9, v/v), respectively. The ratio of the mobile phase B was increased linearly from 5% to 90% over 0.9 min and 90% over the next 1.1 min. HRMS was measured by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) analysis using a Bruker Autoflex Speed. The purities of all compounds tested in biological systems were assessed as being > 95% using elemental analysis or analytical HPLC. Purity data were collected by HPLC with NQAD (Nano Quality Analyte Detector) or Corona CAD (Charged Aerosol Detector). The column was an L-column 2 ODS (30 × 2.1 mm I.D., CERI, Japan) or a Capcell Pak C18AQ (50 × 3.0 mm I.D., Shiseido, Japan) at a temperature of 50 °C and a flow rate of 0.5 mL/min. Mobile phases A and B under a neutral condition were a mixture of 50 mmol/L ammonium acetate, water and acetonitrile (1:8:1, v/v/v) and a mixture of 50 mmol/L ammonium acetate and MeCN (1:9, v/v), respectively. The ratio of mobile phase B was increased linearly from 5 to 95% over 3 min and 95% over the next 1 min. All commercially available solvents and reagents were used without further purification. Yields were not optimized.

Abbreviations are: CD_3OD, deuterated methanol; $CDCl_3$, deuterated chloroform; DIPEA, *N*,*N*'-diisopropylethylamine; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; DMSO-d_6, dimethyl sulfoxide-d_6; EtOAc, ethyl acetate; EtOH, ethanol; HATU, 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate; IPA, isopropyl alcohol; MeCN, acetonitrile; MeI, iodomethane; MeOH, methanol; TFA, trifluoroacetic acid; THF, tetrahydrofuran; *p*-TsCl, *p*-toluenesulfonyl chloride; XPhos Pd G2, [2-(2-aminophenyl)phenyl]-chloro-palladium; dicyclohexyl-[3-(2,4,6-triisopropylphenyl)phenyl]phosphane

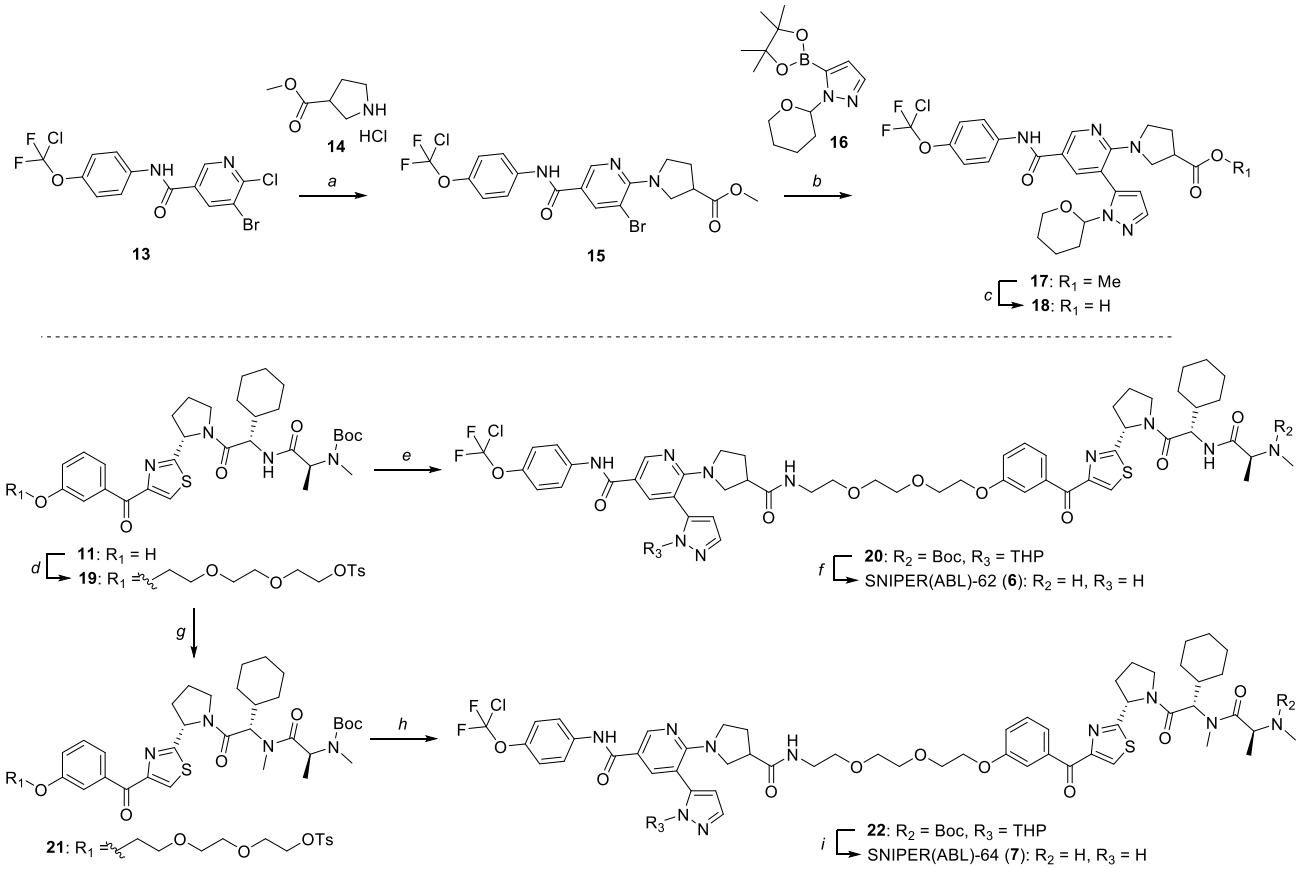
2. Synthetic schemes

Scheme S1. Synthesis of **5**[a]



[a]Reagents and conditions: (a) 2-(2-(2-aminoethoxy)ethoxy)ethanol, HATU, DIPEA, DMF, rt, 79%; (b) *p*-TsCl, DMF, 0 °C, 66% (c) **11**, K_2CO_3, DMF, 60 °C, 59%; (d) TFA, rt, 68%.

Scheme S2. Synthesis of **6** and **7**[a]



[a]Reagents and conditions: (a) **14**, DIPEA, IPA, rt, 130 °C, 65%; (b) **16**, XPhos Pd G2, K_3PO_4, THF, H_2O, 100 °C, 92%; (c) LiOH·H_2O, MeOH, H_2O, rt, 55%; (d) (ethane-1,2-diylbis(oxy))bis(ethane-2,1-diyl) bis(4-methylbenzenesulfonate), K_2CO_3, DMF, 50 °C, 75%; (e) i) NH_3 aq, EtOH, 100 °C; ii) **18**, HATU, DIPEA, CH_3CN, rt, 40%; (f) TFA, rt, 50%; (g) MeI, NaH, DMF, rt, 67%; (h) i) NH_3 aq, EtOH, 100 °C; ii) **18**, HATU, DIPEA, CH_3CN, rt, 51%; (i) TFA, rt, 67%.

3. Experimental procedures

***N*-(2-(2-(2-(3-((2-((2*S*)-1-((2*S*)-2-Cyclohexyl-2-((*N*-methyl-L-alanyl)amino)acetyl)pyrrolidin-2-yl)-1,3-thiazol-4-yl)carbonyl)phenoxy)ethoxy)ethoxy)ethyl)-3-(6-((4-(trifluoromethoxy)phenyl)amino)pyrimidin-4-yl)benzamide (5).**

A mixture of **12** (271 mg, 0.25 mmol) and TFA (1.92 mL) was stirred at room temperature for 30 min. The mixture was diluted with toluene (5 mL) and concentrated *in vacuo*. The residue was partitioned between sat. $NaHCO_3$ aq and EtOAc. The organic layer was separated, washed with sat. $NaHCO_3$ aq and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography (NH silica gel, eluted with 0–10% MeOH in EtOAc) to give the title compound (168 mg, 68 %) as a colorless powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.83–1.01 (3H, m), 1.01–1.29 (7H, m), 1.47–1.77 (6H, m), 1.91–2.12 (2H, m), 2.12–2.27 (4H, m), 2.96 (1H, q, J = 6.6 Hz), 3.42–3.52 (2H, m), 3.54–3.66 (6H, m), 3.73–3.83 (4H, d, J = 5.0 Hz), 4.10–4.16 (2H, m), 4.45–4.52 (1H, m), 5.38 (1H, dd, J = 7.9, 3.0 Hz), 7.21 (1H, dd, J = 7.8, 2.3 Hz), 7.32–7.46 (4H, m), 7.58–7.68 (3H, m), 7.82–7.92 (3H, m), 7.98 (1H, d, J = 7.6 Hz), 8.17 (1H, d, J = 8.1 Hz), 8.47 (1H, s), 8.52 (1H, s), 8.67–8.73 (1H, m), 8.77 (1H, s), 9.92 (1H, s); ^{13}C NMR (151 MHz, DMSO-d_6) δ 13.9, 19.0, 22.0, 24.0, 25.2, 25.4, 25.7, 27.6, 29.3, 30.9, 31.4, 34.2, 34.2, 47.0, 54.0, 58.1, 59.1, 68.8, 68.8, 69.6, 69.8, 102.5, 115.3, 119.3, 119.4, 120.8, 121.0, 121.6, 122.5, 125.3, 128.9, 129.4, 129.8, 135.0, 136.8, 138.1, 139.0, 142.8, 142.8, 152.5, 158.1, 158.2, 160.6, 160.7, 165.8, 170.5, 172.8, 174.3, 185.7; HRMS m/z $(M+Na)^+$ calcd for $C_{50}H_{57}F_3N_8NaO_8S$ 1009.3870, found 1009.3875; purity 99.5% (HPLC).

***N*-(4-(Chloro(difluoro)methoxy)phenyl)-6-(3-((2-(2-(2-(3-((2-((2*S*)-1-((2*S*)-2-cyclohexyl-2-((*N*-methyl-L-alanyl)amino)acetyl)pyrrolidin-2-yl)-1,3-thiazol-4-yl)carbonyl)phenoxy)ethoxy)ethoxy)ethyl)carbamoyl)pyrrolidin-1-yl)-5-(1*H*-pyrazol-5-yl)nicotinamide (6).**

A mixture of **20** (40 mg, 0.03 mmol) in TFA (1 mL) was stirred at room temperature for 30 min. After the mixture was concentrated *in vacuo*, the residue was dissolved in EtOAc-IPA (4:1), washed with sat. $NaHCO_3$ aq and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography (NH silica gel, eluted with 10–30% MeOH in EtOAc) to give the title compound (17 mg, 50 %) as a colorless amorphous solid. ^{1}H NMR (300 MHz, CD_3OD) δ 0.84–1.19 (5H, m), 1.22 (3H, d, J = 6.8 Hz), 1.48–1.85 (7H, m), 2.02–2.42 (8H, m), 2.82–2.97 (1H, m), 3.13 (1H, q, J = 6.9 Hz), 3.21–3.29 (1H, m), 3.35 (3H, d, J = 5.7 Hz), 3.44 (2H, d, J = 7.9 Hz), 3.49–3.57 (2H, m), 3.58–3.66 (2H, m), 3.68–3.78 (2H, m), 3.84–4.02 (4H, m), 4.17–4.26 (2H, m), 4.55 (1H, d, J = 7.2 Hz), 5.46 (1H, dd, J = 7.7, 3.3 Hz), 6.43 (1H, d, J = 2.1 Hz), 7.17–7.31 (3H, m), 7.36–7.46 (1H, m), 7.65–7.82 (5H, m), 8.04 (1H, d, J = 2.5 Hz), 8.29 (1H, s), 8.70 (1H, d, J = 2.5 Hz); ^{13}C NMR (75 MHz, CD_3OD) δ 19.5, 19.7, 25.7, 27.0, 27.1, 27.3, 29.7, 29.7, 30.5, 31.1, 33.2, 34.6, 34.7, 40.6, 41.5, 45.2, 49.0, 50.2, 53.4, 56.9, 60.3, 60.4, 69.0, 70.7, 71.0, 71.4, 72.0, 107.7, 117.0, 119.6, 121.0, 123.1, 123.4, 124.4, 126.9, 130.4, 130.66, 139.4, 139.9, 140.8, 147.6, 149.1, 154.8, 158.9, 160.3, 166.9, 173.0, 174.3, 175.3, 177.2, 188.2; HRMS m/z $(M+H)^+$ calcd for $C_{53}H_{64}ClF_2N_{10}O_9S$ 1089.4235, found 1089.4259; purity 97.5% (HPLC).

***N*-(4-(Chloro(difluoro)methoxy)phenyl)-6-(3-((2-(2-(2-(3-((2-((2*S*)-1-((2*S*)-2-cyclohexyl-2-(methyl(*N*-methyl-L-alanyl)amino)acetyl)pyrrolidin-2-yl)-1,3-thiazol-4-yl)carbonyl)phenoxy)ethoxy)ethoxy)ethyl)carbamoyl)pyrrolidin-1-yl)-5-(1*H*-pyrazol-5-yl)nicotinamide (7).**

A mixture of **22** (70 mg, 0.05 mmol) in TFA (1 mL) was stirred at room temperature for 30 min. After the mixture was concentrated *in vacuo*, the residue was dissolved in EtOAc-IPA (4:1), washed with sat. $NaHCO_3$ aq and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, eluted with 10–40% MeOH in EtOAc) to give the title compound (40 mg, 67%) as a colorless amorphous solid. ^{1}H NMR (300 MHz, CD_3OD) δ 0.90–1.05 (2H, m), 1.19 (2H, brs), 1.25 (3H, d, J = 6.4 Hz), 1.50–1.76 (5H, m), 1.87–1.99 (5H, m), 2.28–2.32 (3H, m), 2.85–2.99 (1H, m), 3.03 (3H, s), 3.23–3.30 (1H, m), 3.42–3.49 (1H, m), 3.52–3.82 (14H, m), 3.90 (3H, t, J = 3.7 Hz), 4.23 (2H, t, J = 4.7 Hz), 4.82–4.87 (1H, m), 5.24 (1H, d, J = 10.6 Hz), 5.39–5.52 (1H, m), 6.46 (1H, s), 7.21–7.34 (3H, m), 7.43 (1H, t, J = 8.1 Hz), 7.67–7.84 (5H, m), 8.06 (1H, d, J = 1.7 Hz), 8.30 (1H, s), 8.72 (1H, s); ^{13}C NMR (151 MHz, CD_3OD) δ 18.6, 24.0, 25.3, 26.7, 26.7, 27.3, 27.4, 29.9, 30.4, 30.4, 30.7, 31.0, 33.3, 34.1, 34.3, 37.1, 40.5, 45.1, 50.1, 53.3, 56.3, 60.3, 60.4, 69.0, 70.5, 70.8, 71.3, 71.8, 107.6, 116.8, 119.5, 120.8, 123.0, 123.3, 124.2, 126.8, 130.3, 130.5, 139.3, 139.8, 140.6, 147.5, 154.6, 158.9, 160.2, 166.9, 170.8, 174.3, 174.3, 175.2, 176.9, 188.2; HRMS m/z $(M+Na)^+$ calcd for $C_{54}H_{65}ClF_2NaN_{10}O_9S$ 1125.4211, found 1125.4174; purity 100% (HPLC).

***N*-(2-(2-(2-Hydroxyethoxy)ethoxy)ethyl)-3-(6-((4-(trifluoromethoxy)phenyl)amino)pyrimidin-4-yl)benzamide (9).**
HATU (1.22 g, 3.20 mmol) was added to a solution of 3-(6-((4-(trifluoromethoxy)phenyl)amino)pyrimidin-4-yl)benzoic acid (**8**)[S1] (1.0 g, 2.66 mmol), 2-(2-(2-aminoethoxy)ethoxy)ethanol (0.716 g, 4.80 mmol) and DIPEA (0.698 mL, 4.00 mmol) in DMF (10 mL) at 0 °C. The mixture was stirred at room temperature for 3 h. The mixture was poured into water and extracted with EtOAc. The organic layer was separated, washed with water, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography (NH silica gel, eluted with 5–15% MeOH in EtOAc) to give the title compound (1.06 g, 79 %) as a colorless foam. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.37–3.50 (6H, m), 3.51–3.66 (6H, m), 4.57 (1H, t, J = 5.3 Hz), 7.31–7.43 (3H, m), 7.64 (1H, t, J = 7.8 Hz), 7.79–7.91 (2H, m), 7.99 (1H, d, J = 7.8 Hz), 8.19 (1H, d, J = 7.9 Hz), 8.48–8.56 (1H, m), 8.67–8.75 (1H, m), 8.77 (1H, s), 9.94 (1H, s); MS m/z $(M+H)^+$ calcd for $C_{24}H_{26}F_3N_4O_5$ 507.2, found 507.3.

2-(2-(2-((3-(6-((4-(Trifluoromethoxy)phenyl)amino)pyrimidin-4-yl)benzoyl)amino)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (10).
p-TsCl (497 mg, 2.61 mmol) was added to a solution of **8** (660 mg, 1.30 mmol) in DMF (13.2 mL) at 0 °C. The mixture was stirred at room temperature for 3 h. The mixture was poured into 1 M HCl aq and extracted with EtOAc. The organic layer was separated, washed with 1 M HCl aq and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, eluted with 50–100% EtOAc in hexane) to give the title compound (567 mg, 66 %) as a colorless oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.40 (3H, s), 3.39–3.67 (10H, m), 4.07–4.13 (2H, m), 7.28–7.40 (3H, m), 7.46 (2H, d, J = 8.1 Hz), 7.63 (1H, t, J = 7.7 Hz), 7.76 (2H, d, J = 8.3 Hz), 7.81–7.92 (2H, m), 7.99 (1H, d, J = 7.8 Hz), 8.19 (1H, d, J = 7.8 Hz), 8.50–8.56 (1H, m), 8.66–8.73 (1H, m), 8.77 (1H, s), 9.94 (1H, s); MS m/z $(M+H)^+$ calcd for $C_{31}H_{32}F_3N_4O_7S$ 661.2, found 661.4.

***tert*-Butyl ((2*S*)-1-(((1*S*)-1-Cyclohexyl-2-oxo-2-((2*S*)-2-(4-(3-(2-(2-(2-((3-(6-((4-(trifluoromethoxy)phenyl)amino)pyrimidin-4-yl)benzoyl)amino)ethoxy)ethoxy)ethoxy)benzoyl)-1,3-thiazol-2-yl)pyrrolidin-1-yl)ethyl)amino)-1-oxopropan-2-yl)methylcarbamate (12).**
K_2CO_3 (71 mg, 0.51 mmol) was added to a solution of **10** (283 mg, 0.43 mmol) and *tert*-butyl ((*S*)-1-(((*S*)-1-cyclohexyl-2-((*S*)-2-(4-(3-hydroxybenzoyl)thiazol-2-yl)pyrrolidin-1-yl)-2-oxoethyl)amino)-1-oxopropan-2-yl)(methyl)carbamate (**11**)[S2] (231 mg, 0.39 mmol) in DMF (5.6 mL) at room temperature. The mixture was stirred at 60 °C under Ar for 5 h. After cooling to rt, the mixture was poured into water and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, eluted with 0–10% MeOH in EtOAc) to give the title compound (274 mg, 59 %) as a colorless foam. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.71–1.14 (6H, m), 1.20–1.26 (3H, m), 1.38 (9H, s), 1.45–1.75 (6H, m), 1.99–2.08 (2H, m), 2.13–2.33 (2H, m), 2.70–2.78 (3H, m), 3.41–3.52 (2H, m), 3.55–3.67 (6H, m), 3.72–3.84 (4H, m), 4.09–4.18 (2H, m), 4.32–4.65 (2H, m), 5.30–5.43 (1H, m), 7.21 (1H, dd, J = 7.9, 2.3 Hz), 7.31–7.38 (3H, m), 7.43 (1H, t, J = 7.9 Hz), 7.57–7.70 (3H, m), 7.81–7.89 (2H, m), 7.93–8.01 (1H, m), 8.10–8.22 (1H, m), 8.47 (1H, s), 8.51–8.55 (1H, m), 8.67–8.73 (1H, m), 8.74–8.77 (1H, m), 9.93 (1H, s); MS m/z $(M+H)^+$ calcd for $C_{55}H_{66}F_3N_8O_{10}S$ 1087.5, found 1087.8.

Methyl 1-(3-Bromo-5-((4-(chloro(difluoro)methoxy)phenyl)carbamoyl)pyridin-2-yl)pyrrolidine-3-carboxylate (15).
To a mixture of 5-bromo-6-chloro-*N*-[4-[chloro(difluoro)methoxy]phenyl]pyridine-3-carboxamide (**13**)[S3] (5.0 g, 12.1 mmol) in IPA (24 mL) was added DIPEA (7.8 g, 60.7 mmol) and methyl pyrrolidine-3-carboxylate HCl salt (**14**) (2.6 g, 15.8 mmol) in a sealed tube at room temperature. The mixture was stirred at 130 °C for 10 h. After cooling to room temperature, the mixture was poured into ice-cold water (30 mL) and adjusted to pH = 5–6 with 1 M HCl aq The aqueous phase was extracted with EtOAc (100 mL × 3). The combined organic phase was washed with brine (100 mL x 3), dried with anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, eluted with 33% EtOAc in petroleum ether) to give the title compound (4.0 g, 65%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.18–2.32 (2H, m), 3.10–3.23 (1H, m), 3.75 (3H, s), 3.82–3.96 (2H, m), 3.99–4.06 (2H, m), 7.19–7.25 (2H, m), 7.63–7.69 (2H, m), 8.02 (1H, s), 8.16 (1H, d, J = 2.0 Hz), 8.55 (1H, s); MS m/z $(M+H)^+$ calcd for $C_{19}H_{18}BrClF_2N_3O_4$ 504.0, 506.0, found 503.9, 505.8.

Methyl 1-(5-((4-(Chloro(difluoro)methoxy)phenyl)carbamoyl)-3-(1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl)pyridin-2-yl)pyrrolidine-3-carboxylate (17).

To a mixture of **15** (4.0 g, 7.9 mmol) and 1-tetrahydropyran-2-yl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrazole (**16**) (3.3 g, 11.9 mmol) in H_2O (10 mL) and THF (100 mL) was added K_3PO_4 (3.4 g, 15.9 mmol) and XPhos Pd G2 (624 mg, 0.8 mmol) in one portion at room temperature under N_2 atmosphere. The mixture was stirred at 100 °C for 16 h. The mixture was concentrated under reduced pressure at 50 °C. The residue was purified by column chromatography (silica gel, eluted with 50% EtOAc in petroleum ether) to give the title compound (4.2 g, 92%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.46–1.58 (2H, m), 1.64–1.75 (1H, m), 1.80–1.90 (1H, m), 2.00–2.17 (3H, m), 2.40–2.56 (1H, m), 2.95–3.08 (1H, m), 3.13–3.34 (1H, m), 3.38–3.50 (2H, m), 3.53–3.60 (1H, m), 3.62–3.78 (4H, m), 3.95–4.05 (1H, m), 4.95–5.09 (1H, m), 6.33 (1H, t, J = 2.0 Hz), 7.22–7.26 (2H, m), 7.61–7.70 (3H, m), 7.75 (1H, s), 7.88 (1H, brs), 8.79 (1H, brs); MS m/z $(M+H)^+$ calcd for $C_{27}H_{29}ClF_2N_5O_5$ 576.2, found 576.1.

1-(5-((4-(Chloro(difluoro)methoxy)phenyl)carbamoyl)-3-(1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl)pyridin-2-yl)pyrrolidine-3-carboxylic Acid (18).

To a mixture of **17** (4.0 g, 6.9 mmol) in methanol (100 mL) and H_2O (10 mL) was added $LiOH{\cdot}H_2O$ (874 mg, 20.8 mmol) in one portion at room temperature. The mixture was stirred at room temperature for 16 h. The mixture was concentrated under reduced pressure at 45 °C. The residue was poured into ice-water (100 mL). The aqueous phase was adjusted to pH 6–7 by citric acid aq (50 %), extracted with EtOAc (100 mL × 3). The combined organic phase was washed with brine (100 mL × 3), dried with anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by prep-HPLC (Phenomenex Gemini, eluted with H_2O (containing 0.05% $NH_3{\cdot}H_2O$ in acetonitrile) to give the title compound (2.1 g, 55%) as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 1.43–1.70 (3H, m), 1.73–2.16 (4H, m), 2.20–2.45 (1H, m), 2.87–3.06 (1H, m), 3.23–3.28 (1H, m), 3.37–3.70 (3H, m), 3.86–4.01 (1H, m), 4.50–4.70 (1H, m), 5.02–5.12 (1H, m), 6.44 (1H, t, J = 2.0 Hz), 7.23–7.29 (2H, m), 7.63 (1H, d, J = 1.6 Hz), 7.74–7.80 (2H, m), 8.00 (1H, brs), 8.80 (1H, d, J = 2.4 Hz); MS m/z $(M+H)^+$ calcd for $C_{26}H_{27}ClF_2N_5O_5$ 562.2, found: 562.2.

2-(2-(2-(3-((2-((2*S*)-1-((2*S*)-2-((*N*-(*tert*-Butoxycarbonyl)-*N*-methyl-L-alanyl)amino)-2-cyclohexylacetyl)pyrrolidin-2-yl)-1,3-thiazol-4-yl)carbonyl)phenoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (19).

A mixture of **11** (599 mg, 1.00 mmol), (ethane-1,2-diylbis(oxy))bis(ethane-2,1-diyl) bis(4-methylbenzenesulfonate) (1.38 g, 3.01 mmol), and K_2CO_3 (277 mg, 2.00 mmol) in DMF (5 mL) was stirred at 50 °C overnight. After cooling, the mixture was diluted with EtOAc, washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, eluted with 50–100% EtOAc in hexane) to give the title compound (668 mg, 75 %) as a colorless oil. ^{1}H NMR (300 MHz, CD_3OD) δ 0.95–1.36 (8H, m), 1.42–1.49 (9H, m), 1.53–1.81 (6H, m), 2.07–2.39 (4H, m), 2.41 (3H, s), 2.68–2.90 (3H, m), 3.54–3.72 (6H, m), 3.79–4.02 (4H, m), 4.09–4.22 (4H, m), 4.46–4.66 (2H, m), 5.43–5.66 (1H, m), 7.22 (1H, ddd, J = 8.3, 2.5, 1.0 Hz), 7.36–7.48 (3H, m), 7.68–7.82 (4H, m), 8.27–8.35 (1H, m); MS m/z $(M+H)^+$ calcd for $C_{44}H_{61}N_4O_{11}S_2$ 885.4, found 884.8.

***tert*-Butyl ((2*S*)-1-(((1*S*)-2-((2*S*)-2-(4-(3-(2-(2-(2-(((1-(5-((4-(Chloro(difluoro)methoxy)phenyl)carbamoyl)-3-(1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl)pyridin-2-yl)pyrrolidin-3-yl)carbonyl)amino)ethoxy)ethoxy)ethoxy)benzoyl)-1,3-thiazol-2-yl)pyrrolidin-1-yl)-1-cyclohexyl-2-oxoethyl)amino)-1-oxopropan-2-yl)methylcarbamate (20).**

The mixture of **19** (70 mg, 0.08 mmol), 28% ammonia aq (1.5 mL, 22.2 mmol) and EtOH (1.5 mL) was heated at 100 °C for 20 min under microwave irradiation. The mixture was poured into water and extracted with EtOAc. The organic layer was separated, washed with sat. $NaHCO_3$ aq and brine, dried over $MgSO_4$, and concentrated *in vacuo* to give the amine as a crude yellow oil. To a solution of the amine, **18** (44.4 mg, 0.08 mmol), and HATU (45.1 mg, 0.12 mmol) in CH_3CN (3 mL) was added DIPEA (0.017 mL, 0.12 mmol) at room temperature. The mixture was quenched with water at room temperature and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over $MgSO_4$. and concentrated *in vacuo*. The residue was purified by column chromatography (NH silica gel, eluted with 0–10% MeOH in EtOAc and then silica gel, eluted with 0–15% MeOH in EtOAc) to give the title compound (40 mg, 40 %) as a colorless gum. ^{1}H NMR (300 MHz, CD_3OD) δ 1.02–1.16 (5H, m), 1.33 (3H, d, J = 7.3 Hz), 1.45 (9H, s), 1.54–1.62 (3H, m), 1.63–1.75 (5H, m), 2.20–2.41 (4H, m), 2.67–2.96 (6H, m), 3.11–3.26 (2H, m), 3.33–3.39 (2H, m), 3.48–3.57 (4H, m), 3.59–3.67 (3H, m), 3.68–3.76 (2H, m), 3.79–4.00 (6H, m), 4.18–4.26 (2H, m), 4.41–4.66 (3H, m), 4.97–5.14 (1H, m), 5.46 (1H, dd, J = 7.6, 3.4 Hz), 6.38–6.43 (1H, m), 7.18–7.30 (4H, m), 7.41 (1H, t, J = 7.9 Hz), 7.58–7.63 (1H, m), 7.68–7.82 (5H, m), 7.96 (1H, brs), 8.29 (1H, d, J = 1.0 Hz), 8.77 (1H, dd, J = 2.4, 1.0 Hz); MS m/z $(M+Na)^+$ calcd for $C_{63}H_{79}ClF_2N_{10}NaO_{12}S$ 1295.5, found: 1295.4.

2-(2-(2-(3-((2-((2*S*)-1-((2*S*)-2-((*N*-(*tert*-Butoxycarbonyl)-*N*-methyl-L-alanyl)(methyl)amino)-2-cyclohexylacetyl)pyrrolidin-2-yl)-1,3-thiazol-4-yl)carbonyl)phenoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (21).

To a solution of **19** (150 mg, 0.17 mmol) and MeI (0.032 mL, 0.51 mmol) in DMF (2 mL) was added 60% NaH in oil (8.1 mg, 0.20 mmol) at room temperature. The mixture was stirred at room temperature for 1 h. The mixture was quenched with water at room temperature and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, eluted with 30–60% EtOAc in hexane) to give the title compound (103 mg, 68%) as a colorless oil. ^{1}H NMR (300 MHz, CD_3OD) δ 0.74–1.05 (3H, m), 1.09–1.22 (2H, m), 1.30–1.36 (2H, m), 1.37–1.44 (2H, m), 1.48 (9H, s), 1.54–1.82 (5H, m), 2.04–2.40 (4H, m), 2.43 (3H, s), 2.83 (3H, s), 3.06 (3H, s), 3.56–3.71 (7H, m), 3.83–3.89 (3H, m), 4.14–4.17 (2H, m), 4.18–4.26 (2H, m), 4.99–5.11 (1H, m), 5.17 (1H, d, J = 10.6 Hz), 5.40–5.51 (1H, m), 7.24 (1H, d, J = 8.1 Hz), 7.39–7.48 (3H, m), 7.70–7.82 (4H, m), 8.33 (1H, s); MS m/z $(M+Na)^+$ calcd for $C_{45}H_{62}N_4NaO_{11}S_2$ 921.4, found 921.2.

***tert*-Butyl ((2*S*)-1-(((1*S*)-2-((2*S*)-2-(4-(3-(2-(2-(2-(((1-(5-((4-(Chloro(difluoro)methoxy)phenyl)carbamoyl)-3-(1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl)pyridin-2-yl)pyrrolidin-3-yl)carbonyl)amino)ethoxy)ethoxy)ethoxy)benzoyl)-1,3-thiazol-2-yl)pyrrolidin-1-yl)-1-cyclohexyl-2-oxoethyl)(methyl)amino)-1-oxopropan-2-yl)methylcarbamate (22).**

A mixture of **21** (100 mg, 0.11 mmol), 28% ammonia aq (1.5 mL, 22.20 mmol) and EtOH (1.5 mL) was heated at 100 °C for 20 min under microwave irradiation. The mixture was poured into water and extracted with EtOAc. The organic layer was separated, washed with sat. $NaHCO_3$ aq and brine, dried over $MgSO_4$ and concentrated *in vacuo* to give amine as a crude yellow oil. To a solution of amine, **18** (62.5 mg, 0.11 mmol), and HATU (63.4 mg, 0.17 mmol) in CH_3CN (3 mL) was added DIPEA (0.024 mL, 0.17 mmol) at room temperature. The mixture was quenched with water at room temperature and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography (NH silica gel, eluted with 60–100% EtOAc in hexane and then silica gel, eluted with 0–15% MeOH in EtOAc) to give the title compound (73 mg, 51 %) as a colorless gum. ^{1}H NMR (300 MHz, CD_3OD) δ 0.84–1.22 (5H, m), 1.31–1.45 (4H, m), 1.48 (9H, s), 1.51–1.80 (9H, m), 1.88–2.01 (3H, m), 2.06–2.45 (6H, m), 2.83 (3H, s), 2.89–3.00 (1H, m), 3.02–3.08 (1H, m), 3.11–3.26 (2H, m), 3.40–4.03 (18H, m), 4.23 (2H, t, J = 4.0 Hz), 4.99–5.19 (3H, m), 5.39–5.48 (1H, m), 6.42 (1H, s), 7.21–7.33 (3H, m), 7.38–7.48 (1H, m), 7.63 (1H, s), 7.69–7.84 (4H, m), 8.30 (1H, s), 8.72–8.86 (1H, m); MS m/z $(M+Na)^+$ calcd for $C_{64}H_{81}ClF_2N_{10}NaO_{12}S$ 1309.5, found 1309.3.

Bioassay section

4. cIAP1, but not XIAP, plays a role in the conjugate 6-induced BCR-ABL protein degradation

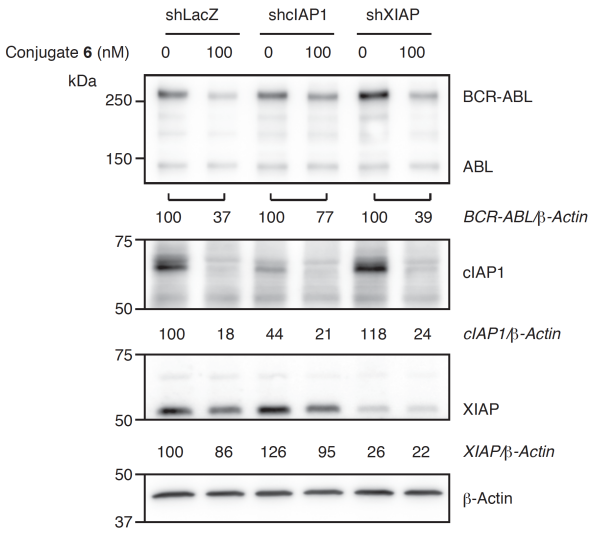


Figure S1. In K562 cell, endogenous cIAP1 or XIAP were depleted by shRNA for 72 h. Then cells were treated with the indicated concentration of conjugate **6** for 6 h. Numbers below the panels represent BCR-ABL/β-Actin, cIAP1/β-Actin, and XIAP/β-Actin ratios normalized by vehicle control as 100.

5. Reduction of cIAP1 protein expression by IAP ligand 4 did not affect the conjugate 6-induced BCR-ABL protein degradation

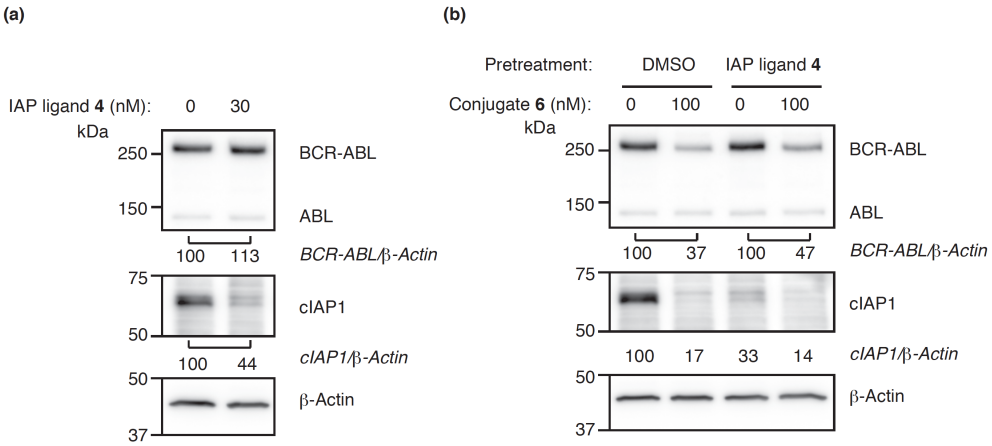


Figure S2. (a) K562 cells were treated with 30 nM of IAP ligand **4** for 3 h. (b) In K562 cell, endogenous cIAP1 protein was degraded by pretreatment with 30 nM of IAP ligand **4** for 3 h. Then the cells were treated with 100 nM of conjugate **6** for 6 h. Numbers below the panels represent BCR-ABL/β-Actin and cIAP1/β-Actin ratios normalized by vehicle control as 100.

6. BCR-ABL degradation of conjugate 6 in KCL-22 cells

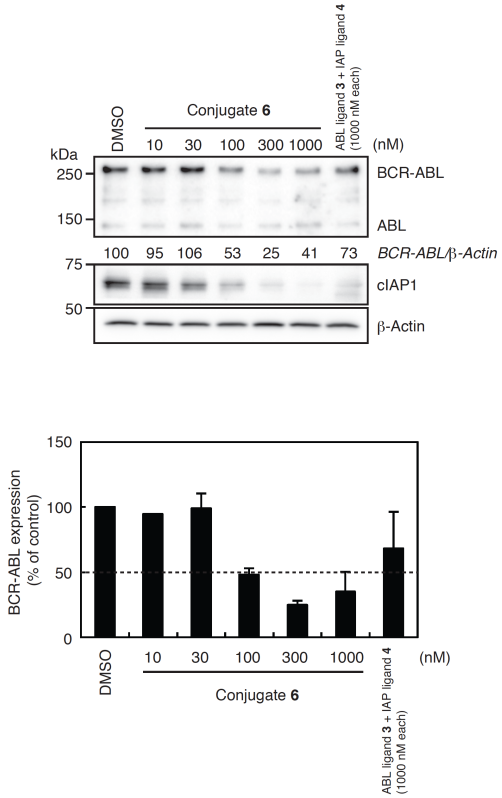


Figure S3. Conjugate **6** shows potent protein knockdown activity in KCL-22 cells. Cells were incubated with the indicated concentration of conjugate **6** or ligands mix (ABL ligand **3** plus IAP ligand **4**) for 6 h. Numbers below the ABL panel represent BCR-ABL/β-actin ratio normalized by the vehicle control as 100. Data in the bar graph are means ± standard deviation (n = 3).

7. IAP ligand 4 did not inhibit proliferation of K562 cells

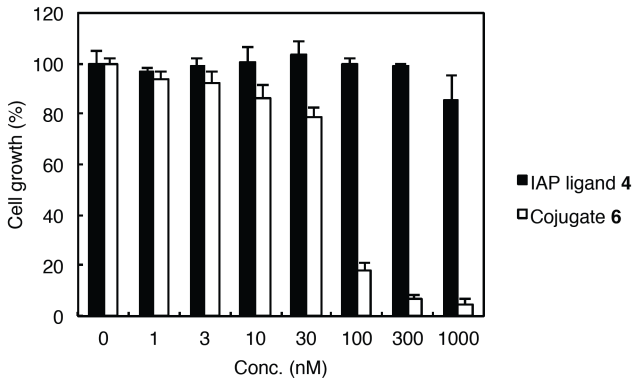


Figure S4. K562 cells were incubated with the indicated concentration of IAP ligand **4** or conjugate **6** for 48 h. Data in the bar graph are means ± standard deviation (n = 3).

8. Binding assay protocols

Materials.

Magnesium chloride and dimethylsulfoxide (DMSO) were purchased from Wako (Osaka, Japan). 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid) (HEPES), biotinylated anti-His antibody and the His-tagged ABL1 protein (full length, ref# P3049) were from Life Technologies (Carlsbad, CA, USA). *o*,*o*'-Bis(2-aminoethyl)ethyleneglycol-*N*,*N*,*N*',*N*'-tetraacetic acid (EGTA) was purchased from Dojindo (Kumamoto, Japan). Brij$^{(R)}$ 35 solution was obtained from Merck Millipore (Billerica, MA, USA). Terbium-labeled streptavidin (Tb-SA) was from Cisbio (Codolet, France). Recombinant His-tagged human XIAP (BIR3, Asn252-Thr356, ref# 895-XB-050) was purchased from R&D Systems (Minneapolis, MN, USA). Recombinant His-tagged human cIAP1 (BIR3, Leu250-Gly350) and cIAP2 (BIR3, Gln238-Ser349) proteins were expressed in *E. coli* and purified using a Ni-NTA column and gel filtration chromatography. The FITC-labeled Smac peptide (FITC-Smac, AVPIAQK(5-FAM)-NH_2)[S4] was synthesized in Scrum (Tokyo, Japan). FITC labeled GNF2 (FITC-GNF2)[S5] was synthesized, as described previously.

Time-resolved FRET (TR-FRET) assay and data analysis.

TR-FRET assays were carried out using 384-well white flat-bottom plates (Greiner Bio-One, Frickenhausen, Germany) and the signal was measured using an EnVision plate reader (PerkinElmer, Waltham, MA, USA). The solution in each well was excited with a laser (λ = 337 nm) reflected by a dichroic mirror (D400/D505 (Perkin Elmer) and fluorescence from terbium (Tb) and BODIPY or FITC were detected through two emission filters (CFP 486 (Perkin Elmer) for Tb, Emission 515 (Perkin Elmer) for FITC). The assay buffer used in this study was 50 mM HEPES (pH 7.2–7.5), 10 mM $MgCl_2$, 1 mM EGTA, 0.1 mM DTT, and 0.01% (v/v) Brij$^{(R)}$ 35. All assays were carried out at room temperature in triplicate or quadruplicate formats.

The percentage of inhibition by test compounds was calculated according to equation (1):

$$\text{Percentage of inhibition} = 100 \times \left(\frac{\mu_H - T}{\mu_H - \mu_L} \right) \quad (1),$$

where T is the value of the wells containing test compounds, and μ_H and μ_L are the mean values of the 0% and 100% inhibition control wells, respectively. The half maximal inhibitory concentration (IC_{50}) and 95% confidence interval of test compounds were calculated by fitting the data with the logistic equation using GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, CA, USA) or XLfit (IDBS, Guildford, UK).

Measurement of inhibitory activity of IAP/peptide interaction.

His-IAP proteins (XIAP, cIAP1, or cIAP2), FITC-Smac, Tb-SA, and the biotinylated anti-His antibody were mixed in the assay buffer and incubated for over 1 h at room temperature before addition to the assay plate. Several concentrations of test inhibitors were dispensed in the assay plate and the protein-probe premix was dispensed to each well. All assays were carried out using 0.6 nM of IAP proteins. The concentrations of FITC-Smac were described as follows: 27 nM for XIAP, 12 nM for cIAP1, and 19 nM cIAP2. The final concentrations of Tb-SA and the biotinylated anti-His antibody were 0.2 and 0.4 nM, respectively. After 1 h incubation at room temperature, the TR-FRET signal was measured using an EnVision plate reader. The values of the 0% and 100% controls were the signals obtained in the presence and absence of IAP proteins, respectively.

Measurement of inhibitory activity of ABL1 inhibitors that bind to the myristoyl binding pocket.

Before addition to the assay plate, 2-fold concentrations of His-ABL1 protein, Tb-SA and the biotinylated anti-His antibody were mixed in the assay buffer and incubated for over 1 h at room temperature. Several concentrations of test inhibitors dissolved in the assay buffer containing 2-fold concentration of FITC-GNF2 were dispensed in the assay plate. Subsequently, an equal volume of protein premix was added to each well. The final concentrations of Tb-SA, biotinylated anti-His, ABL1, and FITC-GNF2 were 0.2, 0.4, 2, and 200 nM, respectively. After 1 h incubation at room temperature, the TR-FRET signal was measured using the EnVision plate reader. The values of the 0% and 100% controls were the signals obtained in the absence and presence of 10 μM ABL-001, respectively.

9. Cellular assay protocols

Reagents.
Tissue culture plastics were purchased from Greiner Bio-One. Inhibitor **23** was purchased from Active Biochem Ltd. (Maplewood, NJ, USA).

Cell culture.
Human CML (K562 and KCL-22), acute T-lymphoblastic leukemia MOLT-4, and T cell leukemia Jurkat cells were cultured in Roswell Park Memorial Institute (RPMI)-1640 medium (Sigma-Aldrich, St. Louis, MO, USA) containing 10% fetal bovine serum (Gibco) and 50 μg/ml kanamycin (Sigma-Aldrich). KCL-22 cells were obtained from Japanese Collection of Research Bioresources (JCRB, Osaka, Japan) Cell Bank (JCRB1317).[S6, S7] The short haipin RNA (shRNA)-mediated gene silencing in K562 cells was carried out, as previously described.[S8]

Western blot analysis.
Cells were collected and lysed in lysis buffer (0.5% TritonX-100, 0.01 M Tris-HCl (pH 7.5), 0.15 M NaCl, Complete Mini protease inhibitor cocktail (Roche Applied Science, indianapolis, IL, USA) and PhosStop phosphatase inhibitor cocktail (Roche Applied Science)). The protein concentration was measured by the BCA method (Thermo Scientific, Rockford, IL, USA) and an equal amount of protein lysate was separated by SDS-PAGE, transferred to polyvinylidene difluoride membranes (Merck Millipore), and analyzed by western blot using an appropriate antibody. The immunoreactive proteins were visualized using the Clarity Western ECL substrate (Bio-Rad, Hercules, CA, USA) and their light emission was quantified with a LAS-3000 lumino-image analyzer (Fuji, Tokyo, Japan). The following antibodies were used: anti-cAbl rabbit polyclonal antibody (pAb) (#2862), anti-XIAP rabbit pAb (#2042), anti-phospho-cAbl rabbit pAb (#3009), anti-STAT5 rabbit pAb (#9363), anti-phospho-STAT5 rabbit pAb (#9359), anti-CrkL mouse monoclonal antibody (mAb) (#3182), and anti-phospho-CrkL rabbit pAb (#3181) (Cell Signaling Technology, Danvers, MA, USA); anti-β-actin mouse mAb (A2228) (Sigma-Aldrich); and anti-cIAP1 goat pAb (AF8181) (R&D systems).

Cell viability assay.
Cell viability was determined using water-soluble tetrazolium WST-8 (4-[3-(2-methoxy-4-nitrophenyl)-2-(4-nitrophenyl)-2*H*-5-tetrazolio]-1,3-benzene disulfonate) for the spectrophotometric assay, according to the manufacturer's instructions (Dojindo). Cells were seeded at a concentration of 5×10^3 cells per well in a 96-well culture plate. After 24 h, the cells were treated with the indicated compounds for 48 h. The WST-8 reagent was added and the cells were incubated for 0.5 h at 37 °C in a humidified atmosphere of 5% CO_2. The absorbance at 450 nm of the medium was measured using the EnVision plate reader.

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