Protein–Protein Interaction

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Discovery of Cell-Permeable Inhibitors That Target the BRCT Domain of BRCA1 Protein by Using a Small-Molecule Microarray**

*Zhenkun Na, Sijun Pan, Mahesh Uttamchandani, and Shao Q. Yao**

Abstract: *BRCTs are phosphoserine-binding domains found in proteins involved in DNA repair, DNA damage response and cell cycle regulation. BRCA1 is a BRCT domain-containing, tumor-suppressing protein expressed in the cells of breast and other human tissues. Mutations in BRCA1 have been found in ca. 50% of hereditary breast cancers. Cell-permeable, small-molecule BRCA1 inhibitors are promising anticancer agents, but are not available currently. Herein, with the assist of microarray-based platforms, we have discovered the first cell-permeable protein–protein interaction (PPI) inhibitors against BRCA1. By targeting the $(BRCT)_2$ domain, we showed compound* ***15a*** *and its prodrug* ***15b*** *inhibited BRCA1 activities in tumor cells, sensitized these cells to ionizing radiation-induced apoptosis, and showed synergistic inhibitory effect when used in combination with Olaparib (a small-molecule inhibitor of poly-ADP-ribose polymerase) and Etoposide (a small-molecule inhibitor of topoisomerase II). Unlike previously reported peptide-based PPI inhibitors of BRCA1, our compounds are small-molecule-like and could be directly administered to tumor cells, thus making them useful for future studies of BRCA1/PARP-related pathways in DNA damage and repair response, and in cancer therapy.*

Phosphorylation-dependent protein–protein interactions (PPIs) control important cell signaling pathways,[1] and are tightly regulated by kinases, phosphatases, and a large number of reader proteins.[2,3] Among them, BRCTs are phosphoserine (*p*Ser)-binding domains commonly found in proteins involved in DNA repair and damage response, and cell cycle regulation.[4] One of the best-known BRCT domain-containing proteins is BRCA1, a tumor-suppressing protein expressed in the cells of breast and other human tissues.[5] BRCA1 is a large, modular protein containing an N-terminal RING domain, a central region and two C-terminal tandem BRCT domains. The BRCT repeats bind to a variety of phosphorylated proteins including BACH1 (a DNA helicase), abraxas, CtIP and others.[6] Together, these PPI complexes play a key role in the DNA damage and repair response. Mutations in BRCA1, including those within its BRCT domains, are found in ca. 50% of hereditary breast cancers.[7] Cancer cells with truncations in the C-terminus of BRCA1 are known to be sensitive to DNA damage-based therapeutics.[5,8] Therefore, cell-permeable, small-molecule PPI inhibitors targeting the BRCT domain of BRCA1 are potential anticancer agents, but are currently not available.[9] The only known BRCA1 inhibitors known in the literature, to our knowledge, are phosphopeptides developed recently by Natarajan and co-workers,[10] but they were not cell-permeable, and required conjugation to cell-penetrating peptides for cellular uptake.[10b]

Each BRCA1 BRCT domain consists of 90–100 amino acids that contain a central four-stranded β-sheet and three α-helices packed along the opposite faces of the sheet (Figure 1a);[11] for the BRCA1/BACH1 complex, the two BRCT repeats stack against each other, in which the N-terminal half of one BRCT domain forms the $p\text{Ser}^{990}$-binding pocket, while the C-terminal half of the same domain provides a hydrophobic binding pocket for Phe^{993} (e.g. the P_{+3} residue in BACH1 phosphopeptide; Figure 1a). Unlike most mainstream pharmaceutical targets, such as enzymes and receptors, which have highly compact and well-defined active sites or pockets, BRCA1 is generally considered "un-druggable".[9] The most viable option for its inhibition is to develop PPI inhibitors that target the BRCT domain. This is however highly challenging, as most small molecules, due to their limited sizes and functional groups, do not provide sufficient enthalpy needed to bind tightly to the much larger protein–protein interface.[12] As such, most known PPI inhibitors are peptide-based and normally possess poor cellular activities (e.g. permeability and stability).[13]

We recently developed a small-molecule microarray (SMM) capable of rapid identification of PPI inhibitors.[14] Few other assays can provide the same type of generality, throughput and sensitivity with minimal consumption of precious proteins.[15,16] By using what we called a fragment-based combinatorial approach,[17] we successfully converted a peptide-based PPI inhibitor of 14-3-3 proteins into cell-permeable small-molecule-like compounds through the construction/screening of peptide-hybrid SMMs.[14] In the current study, we have extended this strategy to the study of BRCA1, and successfully discovered the first cell-permeable inhibitor, compound **15** (Figure 1c). Together with its non-hydrolyzable analog **15a** and the prodrug **15b**, these small-molecule-like dipeptide compounds were shown to possess both in vitro and in-cell antitumor activities via the disruption of BRCA1 $(BRCT)_2$/protein interactions.

The discovery of **15** commenced with microarray determination of the most preferred phosphopeptide sequences

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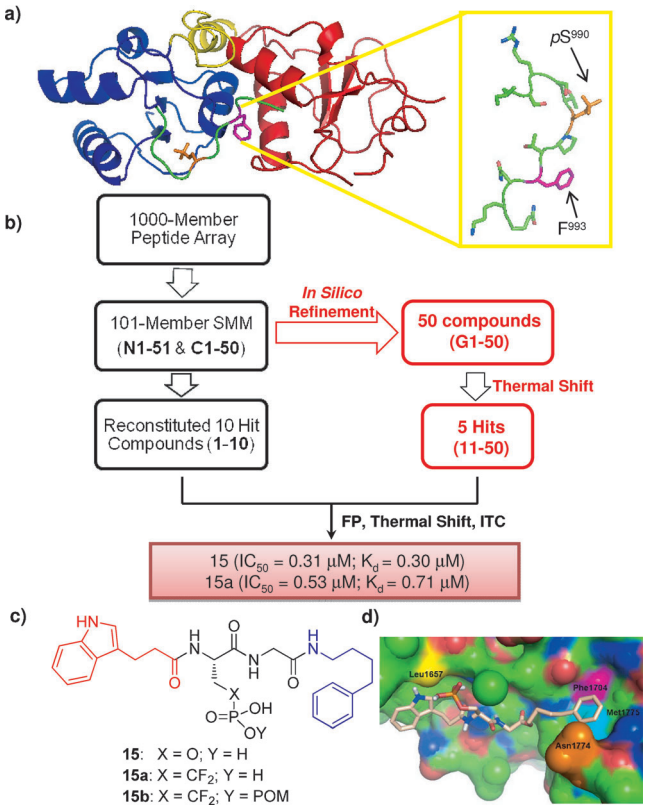


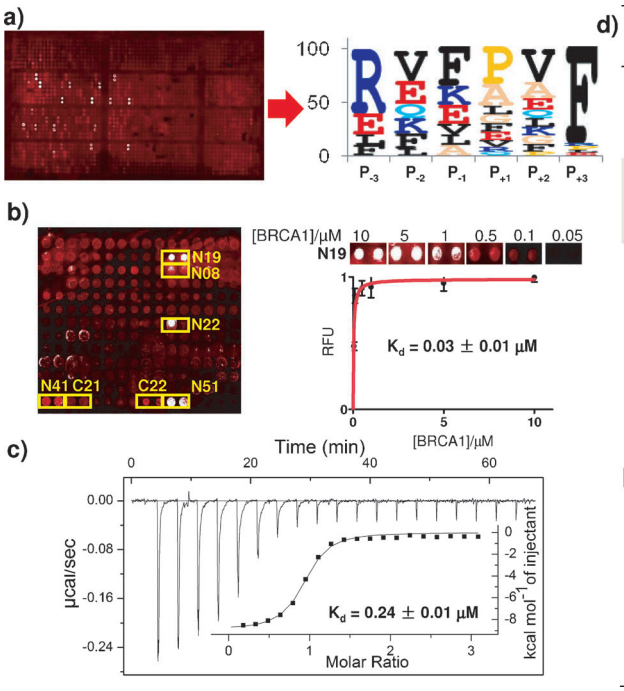
Figure 1. a) Crystal structure of BRCA1 BRCT repeats (shown in ribbons) bound to BACH1 phosphopeptide (PDB ID: 1t29).[11] The BACH1 peptide sequence is ISRST*p*SPTFNKQ, with two critical residues shown in sticks (right inset). b) Workflow of the microarray-assisted discovery of cell-permeable small-molecule-like PPI inhibitors of BRCA1. c) Structures of compounds **15**/**15a**/**15b**. Right: Docked complex of **15** bound to the *p*Ser-binding pocket of BRCA1 $(BRCT)_2$ domain, with critical residues highlighted. POM = pivaloyloxymethyl.

that bind strongly to fluorescently labeled BRCA1 $(BRCT)_2$ (Figure 1b and Figure 2a). By using a previously developed 1000-member combinatorial phosphopetide microarray,[17] we first determined the most preferred BRCA1 $(BRCT)_2$-binding motif as RVF*p*SPVF (Figure 2a, b); this is similar to other reported BRCT-binding sequences and contains a P_{+3} Phe residue as the major binding determinant besides *p*Ser residue.[10,11,18,19] We next converted this phosphopeptide into potential BRCA1 $(BRCT)_2$-binding small-molecule-like compounds (Figure 1b and Figure 2b);[14] by employing the fragment-based combinatorial approach, we synthesized two peptide-hybrid small-molecule libraries (**N1**–**N51** and **C1**–**C50**; see Supporting Information for structures and synthesis). We retained the key *p*Ser residue in RVF*p*SPVF, and systematically replaced the two flanking peptide fragments (RVF and PVF) with commercially available acid and amine building blocks (R_1 and R_2, respectively, in Scheme S1 and Figure 3). Aliphatic/aromatic R_1/R_2 building blocks were chosen for library construction, as they may improve the cell permeability of potential "hits", and based on the X-ray structure of BRCA $(BRCT)_2$ domain, the binding pockets immediately adjacent to the *p*Ser-binding site are mostly hydrophobic.[11] In total, 101 peptide-hybrid small molecules were synthesized, with the N- and C-terminal sub-libraries possessing R_1-*p*SPVF and RVF*p*S-R_2 structures, respectively. All compounds contained a biotin tag for subsequent SMM immobilization. In addition, we carried out in silico refinement of potential binders (see representative docked structure in Figure 1d); results indicate the introduction of a short glycine spacer between *p*Ser and R_2 might project R_2 better into the P_{+3} binding pocket of BRCA1 $(BRCT)_2$. Therefore, an additional 50-member compound library was synthesized (**G1**–**G50**; see Figure 1b, and Figure 3 for synthesis) by using a fixed R_1 acid building block (e.g. $\mathbf{N_{19}}$ shown in Figure 3) with 50 different R_2 amine building blocks (e.g. $\mathbf{C_1}$–$\mathbf{C_{50}}$). Since compounds in **G1**–**G50** do not contain a biotin and were not suitable for SMM immobilization, they were subsequently screened with recombinant BRCA1 $(BRCT)_2$ by using a real-time polymerase chain reaction (PCR)-based thermal shift assay (Figure 1b and Figure 3b).

Upon immobilization of the 101 compounds (**N1**–**N51** and **C1**–**C50**) onto an avidin-functionalized glass slide, the corresponding peptide-hybrid SMM was screened with fluorescently labeled BRCA1 $(BRCT)_2$–GST fusion (Figure 2b); a total of seven potential "hits" were identified (boxed in yellow), representing five N-terminal R_1 building blocks ($\mathbf{N_{08}}$, $\mathbf{N_{19}}$, $\mathbf{N_{23}}$, $\mathbf{N_{41}}$, and $\mathbf{N_{51}}$) and two C-terminal R_2 building blocks ($\mathbf{C_{21}}$ and $\mathbf{C_{22}}$). They were further interrogated by concentration-dependent K_d determination (right images/graph in Figure 2b and S5). Other independent validation experiments were also carried out (Figure 2c, d). With a microarray-measured K_d value of 0.03 μM (IC_{50} = 0.11 μM in FP and K_d = 0.24 μM in ITC), compound **N19** (containing $\mathbf{N_{19}}$) was the strongest $(BRCT)_2$-binding moiety identified in our SMM. It also produced the largest thermal shift (T_m = 53.78 °C). As negative controls, two unrelated BRCT domains were screened on the same SMM, and results indicated none of these seven hits produced any significant binding (Figure S6). We thus concluded that our newly constructed SMM platform was capable of rapid and accurate identification of potential BRCA1 $(BRCT)_2$-binding pharmacophores.

We next carried out reconstitution experiments (Figure 1b);[14] all ten *p*Ser-containing compounds derived from the seven aforementioned hits (i.e. five N-terminal BBs × 2 C-terminal BBs = 10 compounds), **1**–**10**, were synthesized by using the scheme shown in Figure 3. The same scheme was also used to prepare **G1**–**G50** which were $\mathbf{N_{19}}$-optimized, in silico-refined dipeptide compounds. Briefly, 50 different R_2 amines were loaded onto PL-FMP resins by reductive amination (step I). The resulting resins were coupled to Fmoc-Ser[PO(OBzl)-OH]-OH, with or without pre-coupling with Fmoc-Gly-OH (step II). Subsequent coupling of different R_1 acids (step III) to different resins, followed by TFA cleavage (step IV), delivered two sets of compounds, **1**–**10** and **G1**–**G50**, respectively. For **G1**–**G50**, an additional round of thermal shift experiments (step V) was used to rapidly identify five most potent binders of BRCA1 $(BRCT)_2$ that possessed the highest T_m (Figure 3b); these compounds were renamed as **11**–**15**.

The final in vitro validation results of compounds **1**–**15** are summarized in Figure 3c. Two negative controls, **16** (a non-phosphorylated **15**) and **2**–**5** (a previously discovered *p*Ser-



d)

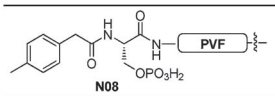
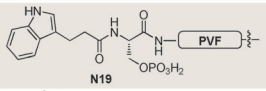
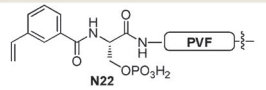
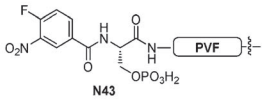
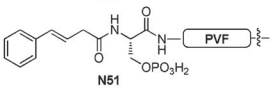
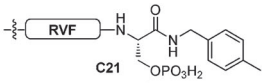
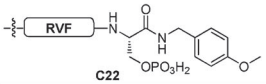
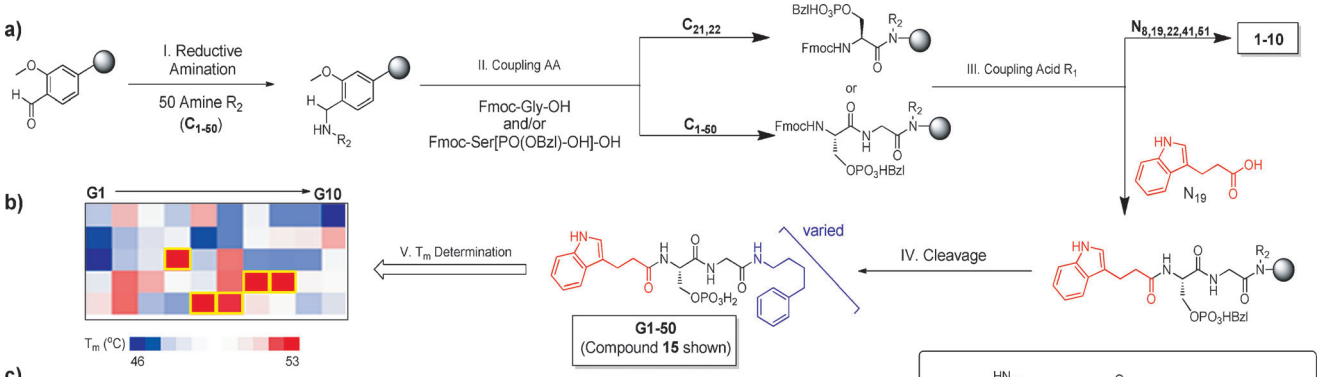
Compound Structure	Array-based K_d [μM]	IC_{50}[a] [μM]	T_m[b] [°C]	K_d[c] [μM]
 N08	0.33 ± 0.02	0.51 ± 0.11	52.14 ± 0.12	0.43 ± 0.02
 N19	0.03 ± 0.01	0.11 ± 0.02	53.78 ± 0.09	0.24 ± 0.01
 N22	0.08 ± 0.02	0.27 ± 0.01	52.56 ± 0.15	0.33 ± 0.02
 N43	0.21 ± 0.04	0.41 ± 0.08	52.11 ± 0.07	0.50 ± 0.03
 N51	0.05 ± 0.02	0.20 ± 0.04	53.22 ± 0.16	0.26 ± 0.02
 C21	0.79 ± 0.09	1.29±0.09	48.91 ± 0.13	1.12 ± 0.04
 C22	0.86 ± 0.08	1.31±0.07	48.56 ± 0.14	1.29 ± 0.08

Figure 2. a) Microarray image of the 1000-member phosphopeptide library screened against Cy5-labeled BRCA1 $(BRCT)_2$. Right: Position-specific scoring matrix (PSSM) representing averaged binding affinity across $P_{+/-1}$, $P_{+/-2}$ and $P_{+/-3}$ positions for each amino acid.[14] The height of each letter represents the weighted contribution of that residue to overall peptide binding. The side chains are colored according to their properties: hydrophobic/aromatic (black), acidic (red), basic (blue), polar (cyan), hydrophilic (green), and small (beige). b) Image of the 101-member peptide-hybrid SMM screened against Cy5-labeled BRCA1 $(BRCT)_2$. Boxed in yellow: Positive hits identified. These hits are summarized in (d). Right: Results of microarray-based K_d determination by using **N19** as a representative. Different concentrations of BRCA1 $(BRCT)_2$ (0–10 μM) were used. RFU = relative fluorescence unit. c) ITC of **N19**/BRCA1 $(BRCT)_2$ interaction, giving the corresponding K_d. d) Summary of hits identified from (b), and their binding affinity towards BRCA1 $(BRCT)_2$ determined by various dose-dependent experiments (microarray K_d determination, [a] fluorescence polarization (FP), [b] real-time PCR-based thermal shift assay, and [c] ITC). See Supporting Information for details.



	IC_{50} [μM][a]	T_m [°C][b]	K_d [μM][c]	Cell Permeability	Hydrolytic Stability /Prodrug Release Rate ($t_{1/2}$)
1-14	0.45-2.23	48.11-52.71	0.39-8.70		
15	0.31 ± 0.07	53.12 ± 0.09	0.30 ± 0.01	695	6 h
15a	0.53 ± 0.05	50.87 ± 0.11	0.71 ± 0.04	713	
15b				704	12 h
16	NA	47.72 ± 0.20	NA		
2-5	> 50	47.93 ± 0.18	43.5 ± 7.8		

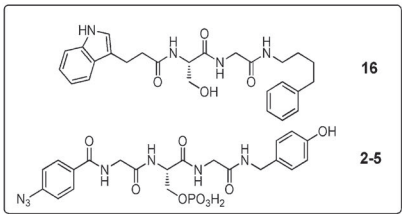
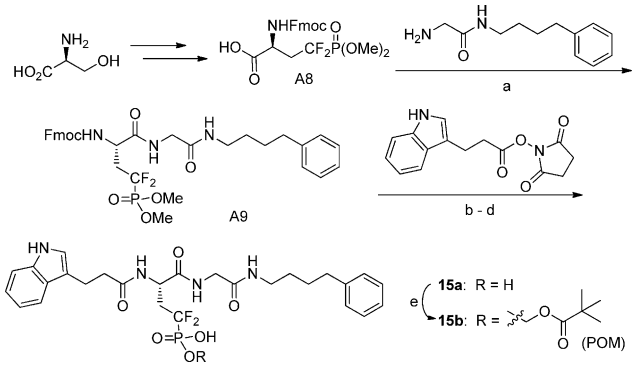


Figure 3. a) Synthesis of the reconstituted compounds, **1**–**10**, and the 50-member library **G1**–**G50** optimized from in silico experiments. b) Heat map showing the relative binding affinity of **G1**–**G50** against BRCA1 $(BRCT)_2$, as determined by T_m measurement using real-time PCR-based thermal shift assay. See Table S3 for complete T_m of compounds. The five most potent binders (**G24**, **G37**, **G38**, **G45**, and **G46**) are highlighted (boxed in yellow), and subsequently renamed as compounds **11**–**15**, respectively. c) Summary of selected compounds, their cell permeability and relatively binding affinities towards BRCA1 $(BRCT)_2$ ([a] FP, [b] thermal shift and [c] ITC). See Supporting Information for complete data (Table S4). Boxed: Structures of two negative controls, **16** and **2**–**5**. NA = not available (e.g. inhibition was too weak to be detected). Hydrolytic stability/prodrug release rate of **15**/**15b** (in $t_{1/2}$) are shown as well.

containing 14-3-3 PPI inhibitor[14]), were tested concurrently. All binding experiments (FP, T_m determination and ITC) showed **1**–**15** were relatively potent binders of BRCA1 $(BRCT)_2$, with **15** being the best ($IC_{50} = 0.31\ \mu M$, $T_m = 53.12\,°C$, $K_d = 0.30\ \mu M$). On the other hand, **16** and **2**–**5** showed no and weakly detectable binding to BRCA1 $(BRCT)_2$, respectively. These results indicate that the strong and specific affinity of **15** toward BRCA1 $(BRCT)_2$ did not originate from the *p*Ser moiety alone. Our docking results further supported this conclusion (Figure 1d); in addition to the extensive hydrogen bonds expected between *p*Ser in **15** and $(BRCT)_2$, the indole ring of $\mathbf{N_{19}}$ was engaged in π–π interaction with Phe^{1662}, and hydrogen-bonded with the backbone amide of Leu^{1657}. The aromatic ring of $\mathbf{C_{37}}$ also stacked nicely against a hydrophobic pocket formed by the side chains of Phe^{1704}, Asn^{1774} and Met^{1775} from the BRCA1 $(BRCT)_2$ domain.

Compound **15** contains a *p*Ser moiety, which could be readily dephosphorylated by endogenous phosphates (to give **16**). In fact, an in vitro hydrolytic stability assay showed that, upon treatment with mammalian lysates, around half of **15** was converted to **16** in 6 h (Figure 3c and S7). We therefore synthesized the non-hydrolyzable version, **15a**, by replacing *p*Ser in **15** with α,α-difluoromethylenephosphoserine.[20,21] In addition, a prodrug version, **15b**, was synthesized by introducing a pivaloxymethyl (POM) group to the phosphonate moiety in **15a** (Scheme 1). Such strategy may improve



Scheme 1. Reagents and conditions: a) 2-amino-*N*-(4-phenylbutyl)acetamide, PyBOP, DIEA, 40%; b) DBU; c) TMSI; d) 2,5-dioxopyrrolidin-1-yl-2-(4-azidobenzamido)acetate, Et_3N, 26% over 3 steps; e) POMCl, DIEA, 19% (42% conversion). See Scheme S4 for details. PyBOP = benzotriazolyl-1-oxy-tripyrrolidino-phosphonium hexafluorophosphate, DIEA = ethyldiisoproplyamine, DBU = 1,5-diazabicyclo[5.4.0]undec-5-ene, TMSI = trimethylsilyliodide.

a drug's cell permeability and intracellular stability.[22] The synthesis of **15a**/**15b** is summarized in Scheme 1. The key intermediate, difluoromethylenephosphoserine **A8**, was prepared by modifications of previously reported procedures.[20] Subsequent PyBOP coupling of 2-amino-*N*-(4-phenyl)acetamide furnished **A9**, which was followed by deprotection steps and coupling with the NHS-activated $\mathbf{N_{19}}$ acid building block to give the desired **15a**. Subsequent treatment of **15a** with POM-Cl afforded **15b**.

As expected, **15a** was completely stable toward mammalian lysate treatment under similar hydrolytic stability assay conditions (Figure S7), while maintaining comparable in vitro binding affinity to BRCA $(BRCT)_2$ (Figure 3c). All three compounds were cell-permeable as determined by a standard MDCK cell permeability assay. Prodrug **15b** was shown to possess marginally improved cell permeability, and could be readily converted to **15a** by incubation with mammalian cell lysates (Figure S7). We first investigated whether **15** and **15a** compete with cellular proteins for binding to BRCA1 $(BRCT)_2$ in complex cellular environments. In vitro pull-down experiments were performed by using HeLa cell lysates with bead-immobilized BRCA $(BRCT)_2$–GST fusion (Figure 4a); pre-incubation of the bead with **15** or **15a** (**16** and DMSO (dimethyl sulfoxide) were used as negative controls) showed both compounds potently disrupted BRCA1/BACH1 interaction in a dose-dependent manner. The same effect was not observed with **16** even at a high concentration (50 μM). BRCA1 plays a key role in the repair of double-strand DNA breaks (DSBs),[5] and its genetic knockdown is known to cause partial inhibition (ca. 60%) in DNA homologous recombination (HR).[23] In our experiments (Figure 4b and S8), compounds **15**/**15a**/**15b**, but not **16**, were found to inhibit HR activity in HeLa cells; cells treated with either **15a** or **15b** (50 μM) resulted in ca. 55% reduction in HR activity, similar to that observed in cells transfected with siRNA of BRCA1 (100 nM). These findings suggest **15a**/**15b** could effectively inhibit HR activity by binding to BRCA1$(BRCT)_2$, and functionally mimic genetic knockdown of BRCA1. As expected, **15** possessed lower cellular activity (e.g. 25% reduction in HR activity under similar conditions), likely due to its intracellular instability. PARP, or poly-ADP-ribose polymerase, is chiefly involved in the repair of single-strand DNA breaks (SSBs).[24] Small-molecule active-site inhibitors of PARP are promising chemosensitizers, with many of them including Olaparib already in clinical trials as potential anticancer drugs.[25] Recent studies showed that synergistic inhibition of PARP/BRCA1 (a process referred to as "synthetic lethality"), is highly effective in cancer therapy.[26] With the discovery of compounds **15**/**15a**/**15b**, we hypothesized their ability to serve as a PPI inhibitor of BRCA1 might be exploited to render cancer cells more susceptible to PARP inhibition. We therefore subjected HeLa cells to combination drug treatments of **15**/**15a**/**15b** and Olaparib in a cell-based XTT anti-proliferation assay (Figure 4c and S9); we found both **15b** and Olaparib alone caused cell death in a concentration-dependent manner, but in combination, the effect appeared much more significant (Figure 4c, left). For results on **15**/**15a**, see the Supporting Information. We further confirmed the synergistic effects of these combination drug treatments, by calculating the corresponding combination index (CI) values (Figure 4c, right); CI values of < 1 at all tested inhibition levels clearly indicate synergy, not additivity, of these compounds. Further evidence was obtained by carrying out similar experiments with Etoposide (a DNA topoisomerase II inhibitor), another drug previously shown to have synergistic effects on BRCA1-mutated cancer cells (Figure S9b).[27] Similar effects were also observed with BRCA1-knocked down HeLa cells treated with Olaparib or

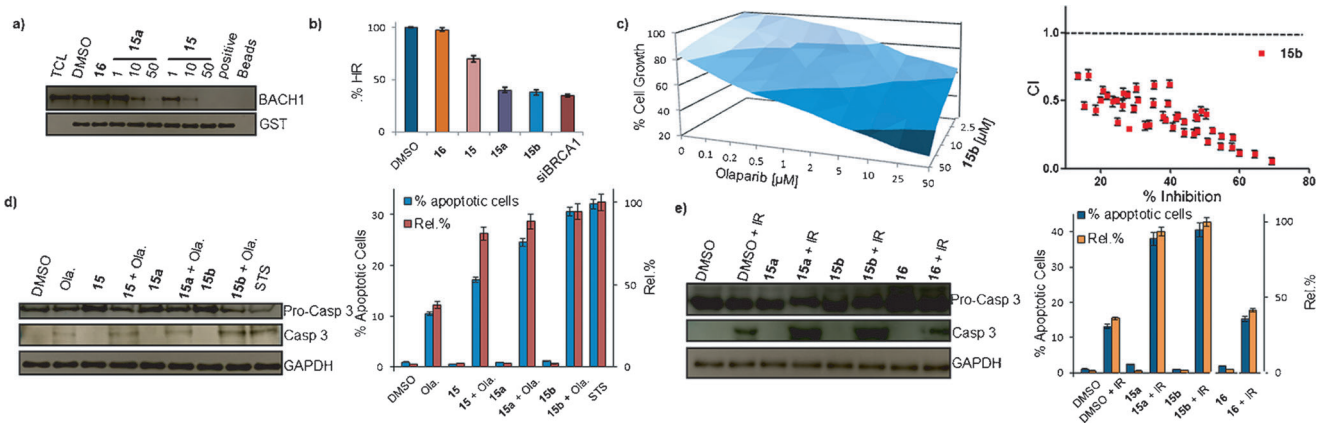


Figure 4. a) In vitro competitive binding of **15**/**15a** with cellular proteins for BRCA1 $(BRCT)_2$. Each compound (DMSO, **15**, **15a**, **16**, positive control peptide; 50 μM each unless otherwise indicated) were pre-incubated with bead-immobilized GST-BRCA1-$(BRCT)_2$. Then HeLa cell lysates were added. After binding/washing, bead-bound proteins were separated by SDS-PAGE and immunoblotted with anti-BACH1 antibody (anti-GST was used as a loading control). Positive control peptide = SRST*p*SPTFNK-$CONH_2$. TCL = total cell lysates. For **15** and **15a**, three concentrations (1, 10, and 50 μM) were used. b) HeLa cells were treated with a compound (50 μM), then transfected with the kit to detect the HR level. Positive control was siBRCA1-transfected cells and negative control was DMSO. c) XTT anti-proliferation results of HeLa cells treated with different amounts of Olaparib and **15b** for 24 h. Right: the corresponding CI plot. d) Cell death/apoptosis analysis of HeLa cells treated with combination drugs as shown in (c). 50 μM of each compound was used. STS (200 nM)- and siBRCA1 (100 nM)-treated cells were used as positive controls. Treated cells were analyzed by Western blotting with anti-pro-caspase 3 and anti-caspase 3 antibodies (anti-GAPDH = loading control) (left) and FACS/caspase-3 enzymatic activity analysis (right). % apoptotic cells were determined by FACS using Annexin-V kit. Rel%: relative caspase-3 activity determined by Ac-DEVD-AMC. e) γ-Irradiation induced apoptosis of HeLa cells with and without treatment of 50 μM of each compound (**15a**, **15b**, **16**; 24 h). Cells were then treated with 20 Gy by γ-irradiation. Cells were subsequently analyzed as shown in (d). See Supporting Information for details.

Etoposide (Figure S9c). All these lines of evidence clearly showed our newly developed PPI inhibitors of BRCA1, when combined with potential drugs such as Olaparib, could much more effectively kill tumor cells. We further showed that cell death caused by these combination drug compounds went through the apoptosis pathway via activation of caspase-3 activity (Figure 4d and S10); while Olaparib treatment alone caused cell death with some degrees of apoptosis, as determined by Western blotting (WB), FACS analysis and caspase-3 enzymatic assays, combination treatments with **15**/**15a**/**15b** significantly elevated this effect. In all experiments, the cellular activities of our compounds were in the expected order of **15b** ≈ **15a** ≫ **15**, clearly reflecting the effect of improved cellular stability of **15a**/**15b**. Finally, siRNA-knockdown of BRCA1 was previously shown to render tumor cells hypersensitive to apoptosis in response to DNA damage.[28,29] We wondered if inhibition of $BRCA1(BRCT)_2$ by **15a**/**15b** (a chemical "knockdown" of BRCA1) would have similar effects as well. As shown in Figure 4e (and Figure S10), while some degrees of apoptosis and caspase-3 activation were observed by HeLa cells exposed to γ-irradiation (20 Gy), pre-treatment of these cells with either **15a** or **15b**, but not **16**, significantly elevated these effects.

In conclusion, with the assist of microarray-based screening platforms, we have successfully discovered the first-ever cell-permeable PPI inhibitors that target the BRCT domain of BRCA1 both in vitro and in cell-based experiments. Improvements were made from the initial hit compound **15** by making its corresponding non-hydrolyzable **15a** and the prodrug **15b**, both of which were shown to possess reasonable activities in inhibiting BRCA1-associated cellular events. As novel small-molecule-like BRCA1 inhibitors, both **15a** and **15b** showed synergistic inhibitory effects on tumor cells when used in combination with Olaparib and Etoposide. Further evidence showed the effect of combination drug treatments by these compounds went through the apoptosis pathway. Chemical knockdown of BRCA1 by these compounds in tumor cells rendered them hypersensitive to γ-irradiation. Unlike previously reported, peptide-based BRCA1 PPI inhibitors which were cell-impermeant,[10] our compounds possess small-molecule-like properties and could be directly administered to tumor cells. This feature will make them useful in future studies of BRCA-related pathways in DNA damage and repair, and in cancer therapy.

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