Protein–Protein Interactions Hot Paper

DOI: 10.1002/ange.201410678

A Small-Molecule Protein–Protein Interaction Inhibitor of PARP1 That Targets Its BRCT Domain**

*Zhenkun Na, Bo Peng, Shukie Ng, Sijun Pan, Jun-Seok Lee, Han-Ming Shen, and Shao Q. Yao**

Abstract: *Poly(ADP-ribose)polymerase-1 (PARP1) is a BRCT-containing enzyme (BRCT = BRCA1 C-terminus) mainly involved in DNA repair and damage response and a validated target for cancer treatment. Small-molecule inhibitors that target the PARP1 catalytic domain have been actively pursued as anticancer drugs, but are potentially problematic owing to a lack of selectivity. Compounds that are capable of disrupting protein–protein interactions of PARP1 provide an alternative by inhibiting its activities with improved selectivity profiles. Herein, by establishing a high-throughput microplate-based assay suitable for screening potential PPI inhibitors of the PARP1 BRCT domain, we have discovered that (±)-gossypol, a natural product with a number of known biological activities, possesses novel PARP1 inhibitory activity both in vitro and in cancer cells and presumably acts through disruption of protein–protein interactions. As the first known cell-permeable small-molecule PPI inhibitor of PAPR1, we further established that (−)-gossypol was likely the causative agent of PARP1 inhibition by promoting the formation of a 1:2 compound/PARP1 complex by reversible formation of a covalent imine linkage.*

Poly(ADP-ribose)polymerase-1, or PARP1, is a 113 kDa nuclear protein mainly involved in the repair of single-stranded breaks (SSBs) and double-stranded breaks (DSBs) of DNA. It is also known to participate in a variety of cellular functions, including transcriptional regulation, chromosome stability, cell division, differentiation, and apoptosis.[1] PARP1 belongs to a family of at least 18 human proteins, most of which share highly homologous catalytic domains.[2] As the most actively pursued target for cancer treatment amongst the PARP family, many small-molecule inhibitors against PARP1 have been developed, some of which, including Olaparib and BMN-673, are already in advanced stages of clinical trials.[3] With the exception of some coumarins and indoles, all known PARP1 inhibitors are nicotinamide analogues that bind in the NAD^+ binding pocket of the catalytic domain (Figure 1)[3,4] and thus possess promiscuous inhibitory activity. There is an urgent need to discover highly potent and selective compounds that inhibit the enzymatic activity of PARP1, but not those of other PARP proteins.[5]

In addition to the catalytic domain (containing HD and ART subdomains; Figure 1 a), five other domains are present in PARP1: three zinc-binding domains (Zn1, Zn2, and Zn3) and the WGR domain, which collectively participate in binding to damaged DNA, and the BRCT (BRCA1 C-terminus) domain.[6] BRCT is a well-known phosphoryla-

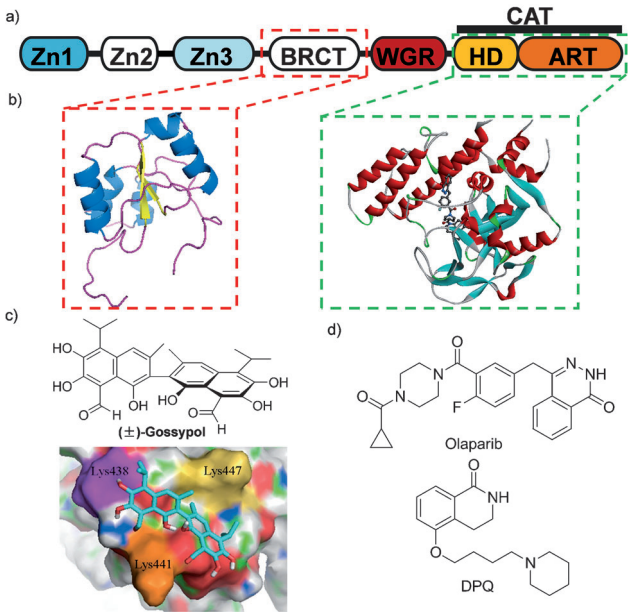

Figure 1. a) Domain architecture of human PARP1. b) NMR structure of the PARP1 BRCT domain (left; PDB ID: 2COK),[8] and X-ray structure of a complex of the PARP1 catalytic domain with Olaparib (right; PDB ID: 4HHY). c) Structure of (±)-gossypol (only the (−)-form is shown). Bottom: (−)-Gossypol bound to the PARP1 BRCT domain. d) Representative examples (Olaparib and DPQ) of PARP1 active-site inhibitors.

[*] Z. Na, B. Peng, S. Ng, S. Pan, Prof. Dr. S. Q. Yao
Department of Chemistry, National University of Singapore
3 Science Drive 3, Singapore 117543 (Singapore)
E-mail: chmyaosq@nus.edu.sg
Homepage: http://staff.science.nus.edu.sg/~syao
Prof. Dr. H.-M. Shen
Department of Physiology, National University of Singapore
16 Medical Drive, Singapore 11794 (Singapore)
Prof. Dr. J.-S. Lee
Molecular Recognition Research Center
Korea Institute of Science and Technology (KIST)
39-1 Hawolgok-dong, Seoul, 136-791 (Republic of Korea)
and
Department of Biological Chemistry
University of Science and Technology (UST)
113 Gawhank-ro, Yuseong-gu, Daejeon, 305-333 (Republic of Korea)

[**] Funding was provided by the National Council (CBRG/0038/2013) and the Ministry of Education (MOE2012-T2-1-116 and MOE2012-T2-2-051). The mass spectrometry work was supported by intramural funding from KIST (2Z04070/2E24860-2E25192). We also thank Bob London (NIH) and Daiwen Yang (NUS) for helpful discussions, and Dr. H. Schuler (KI) for cat-PARP1 plasmid constructs. BRCT = BRCA1 C-terminus, PARP1 = poly(ADP-ribose)-polymerase-1.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201410678.

tion reader domain, often found in proteins involved in DNA repair and the cell cycle.[7] The PARP1 BRCT domain consists of 90–100 amino acids with a central four-stranded β-sheet and three α-helices packed along the opposite faces of the sheet (Figure 1b, left).[8] It is a phosphoserine (pSer) binding domain and mediates important protein–protein interactions (PPIs) with a number of other proteins, such as XRCC1, Hsp70, and OGG1.[1,9] The formation of macromolecular complexes between PARP1 and these proteins is crucial for the efficient repair of damaged DNA and cell signaling. Interestingly, PARP1 is one of only two presently known BRCT-containing enzymes in the human PARP protein family.[10] We previously showed that by targeting the PPI interface, it is possible to develop small-molecule cell-permeable inhibitors of phosphorylation readers, such as 14-3-3 and BRCA1.[11] Such inhibitors are attractive leads for drug discovery and generally possess better selectivity profiles than active-site inhibitors.[12] The development of PPI inhibitors is, however, highly challenging.[13] Herein, by establishing the first high-throughput assay suitable for screening potential PPI inhibitors of the PARP1 BRCT domain, we have discovered that (±)-gossypol, a natural product with a number of previously reported biological activities (Figure 1c),[14] possesses potent and novel PARP1 inhibitory activity through disruption of BRCT-mediated protein–protein interactions, both in vitro and in cancer cells. We further established that the (−)-gossypol isomer was likely the causative agent by promoting the formation of a 1:2 compound/BRCT complex through reversible formation of covalent imine linkages.

To discover small-molecule PPI inhibitors against the PARP1 BRCT domain, we adopted the workflow shown in Figure 2a. To the best of our knowledge, the consensus phosphopeptide-binding sequence to PARP1 BRCT is not known,[15] and no high-throughput screening (HTS) assay is available to identify compounds capable of competing for binding to this domain.[7] We therefore first determined the most preferred pSer-containing heptapeptide that binds to PARP1 BRCT by screening the fluorescently labeled recombinant protein with a previously reported, 1000-member universal phosphopeptide microarray (Step I).[16] We determined the most preferred PARP1 BRCT domain binding peptide as LRFpSVFF (Figure 2b), which, aside from the pSer residue, contains a P_{+3} Phe residue as the other major binding determinant. Next, we further optimized this sequence by constructing a 115-member positional scanning (PS) heptapeptide microarray (Step II); all six residues in LRFpSVFF (except pS) were individually substituted with each of the 20 naturally occurring amino acids.[17] Position-specific scoring matrix (PSSM) analysis of the microarray screening results revealed an equally strong PARP1 BRCT binding sequence, LRFpSRFF. Further isothermal titration calorimetry (ITC) experiments indicate that both peptides bound to the recombinant PARP1 BRCT domain with equal affinities ($K_d = 4.86 \pm 0.21$ μM), which is moderate but sufficient for subsequent fluorescence polarization (FP) experiments (Step III). To convert LRFpSVFF into a FP-compatible PARP1 BRCT binding probe for HTS of PPI inhibitors, we attached a fluorescent dye to the N-terminus of the peptide (Figure 2d,e); the final peptide exhibited a good protein-binding curve in a microplate-based FP assay with an improved K_d value of 1.1 ± 0.11 μM. This thus represents the first fluorescence-based, HT-ready microplate assay for the screening of potential PPI inhibitors against PARP1. We next carried out a small-scale screening assay by using a relatively small compound library, which consisted of approximately 150 commercially available natural products and about 280 in-house compounds. Many of the in-house compounds were candidate hits identified in previous screening efforts against other reader proteins.[11,18] We optimized the FP assay in a 384-well microplate format, which may be adopted for high-throughputs screening with much larger compound libraries in the future (Step III). Upon identification of candidate hits, they were further validated by in vitro quantitative binding assays (FP, thermal shifts, ITC) and in situ cell-based assays (Step IV).

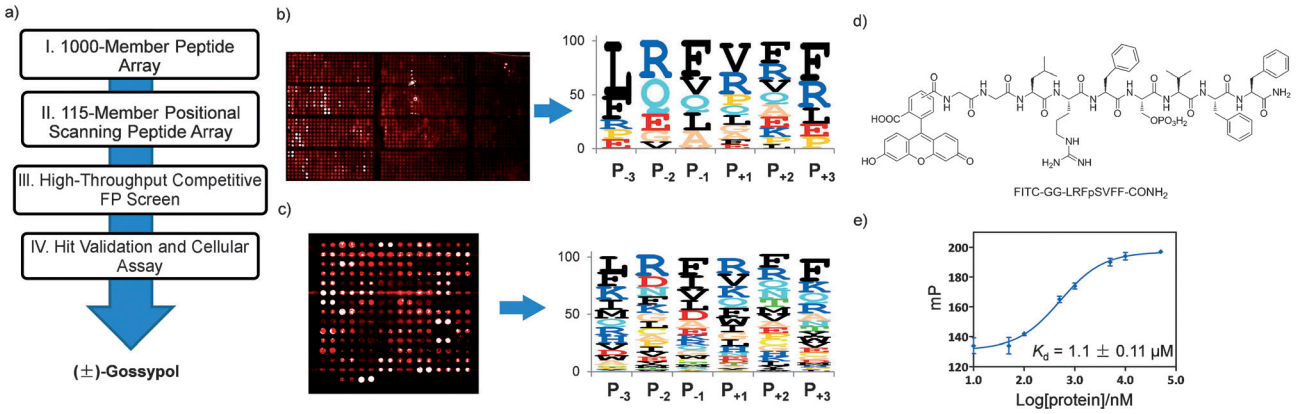

Figure 2. a) Workflow leading to the discovery of the first small-molecule cell-permeable PPI inhibitor of PARP1, (±)-gossypol. b) Image of the 1000-member universal phosphopeptide microarray screened against Cy5-labeled PARP1 BRCT protein. Right: PSSM graph representing the averaged binding affinity across different positions for each amino acid.[16] The height of each letter represents the weighted contribution of that residue to overall peptide binding. c) Image of the 115-member PS peptide microarray screened against Cy5-labeled PARP1 BRCT. Right: PSSM graph. d) Structure of the FITC-labelled consensus peptide that binds to PARP1 BRCT and was used in the fluorescence polarization assay. e) FP response curve, showing strong binding between the peptide and PARP1 BRCT ($K_d = 1.1 \pm 0.11$ μM). mP = millipolarization unit.

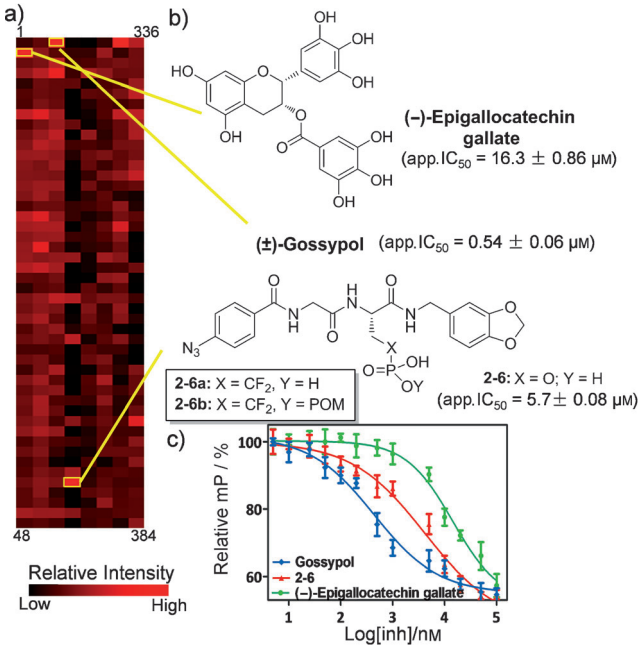

Figure 3. a) Heat map summarizing the screening results of the 384-member small-molecule library (screening concentration: 20 μM) against the PARP1 BRCT protein in an FP assay. See the Supporting Information, Section 2 for compound IDs and structures. b) Structures of selected hits and the corresponding apparent IC_{50} values. c) Concentration-dependent IC_{50} values determined by an FP assay (1 h incubation).

From the FP screening results (Figure 3), several hits were positively identified and taken further for quantitative in vitro binding analysis. Representative results are shown in Figure 3b and c; to our pleasant surprise, the natural product (±)-gossypol emerged as the most potent hit in competing for binding to the PARP1 BRCT domain, with an apparent IC_{50} value of 0.54 ± 0.06 μM ($K_d = 4.11 \pm 0.21$ μM from ITC); (±)-gossypol was at least ten times more potent than its closest counterpart, **2-6. 2-6** is an in-house compound previously shown to bind to 14-3-3 with moderate activity.[11a] Interestingly, two other pSer-containing small molecules, **2-5** and **15** (see the Supporting Information, Section 2 for the structures), which are previously identified potent PPI inhibitors of 14-3-3 and BRCA1, respectively, showed no appreciable binding towards PARP1 BRCT.[11] Further attempts to structurally optimize **2-6** by making the corresponding non-hydrolysable version, **2-6a**, which contains an α,α-difluoromethylenephosphoserine in place of pSer, and the prodrug version, **2-6b**, which contains a pivaloxymethyl (POM) group, failed; none of these three compounds showed any acceptable cellular activities and were not pursued further.

We first investigated whether (±)-gossypol competed with cellular proteins for binding to PARP1 BRCT in a complex proteome. In vitro pull-down experiments were performed by using HeLa cell lysates with Ni-NTA bead-immobilized $(His)_6$-PARP1-BRCT fusion (Figure 4a); pre-incubation of the bead with (±)-gossypol showed that the compound potently disrupted the interaction of the PARP1 BRCT domain with two well known PARP1-interacting proteins, Hsp70 and OGG1.[9b,c] In contrast, Olaparib, a potent PARP1 active-site inhibitor ($IC_{50} = 3.8$ nM),[19] showed no such effect even at a high dosage (50 μM). Further evidence of PARP1 inhibition by (±)-gossypol by specific interaction with its BRCT domain was gathered by using an in vitro enzymatic assay with both full-length PARP1 (FL-PARP1) and the catalytic domain of PARP1 (cat-PARP1), and ITC binding experiments with the PARP1 BRCT domain (Figure 4b and Figure S4); results clearly show that unlike DPQ, (±)-gossypol inhibited the enzymatic activity of FL-PARP1 ($IC_{50} = 1.14 \pm 0.09$ μM) by binding exclusively to the enzyme's BRCT domain ($K_d = 4.11 \pm 0.21$ μM). We next evaluated whether these in vitro effects could be repeated in cancer cells. Lysates from HeLa cells treated with (±)-gossypol were immunoprecipitated with an anti-PARP1 antibody, followed by SDS-PAGE and Western blotting (WB) analysis (Figure 4c). Dose-dependent inhibition of the PARP1/Hsp70 association was clearly detected, indicating that (±)-gossypol blocked protein–protein interactions of PARP1 inside mammalian cells as well. To ascertain whether this led to direct inhibition of the endogenous PARP1 activity, we evaluated the poly(ADP-ribosyl)ation (PAR) formation in HeLa cells under oxidative stress.[20] Compound-treated HeLa cells were subjected to H_2O_2 for different periods of time, followed by WB detection of PAR polymer formation (Figure 4d); a significant decrease in PAR formation was observed in both DPQ- and (±)-gossypol-treated cells. Similar effects were observed in an imaging-based immunofluorescence assay (Figure S16); a significantly smaller number of PAR-positive nuclei were detected in cells knocked down by siRNA of PARP1 or treated with (±)-gossypol or DPQ. Recent studies showed that the combination treatment of cancer cells with PARP1 inhibitors and other DNA alkylating agents such as Temozolomide is highly effective in killing tumor cells.[21] With the ability of (±)-gossypol to functionally mimic the genetic knockdown of PARP1, we hypothesized that synergistic inhibition of growth would be expected for HeLa cells treated with both (±)-gossypol and Temozolomide, and this was indeed the case (Figure 4e). Calculated combination index (CI) values of < 1 at all tested inhibition levels clearly indicate the synergy of these compounds. Finally, siRNA knockdown of BRCA1 was previously shown to render tumor cells hypersensitive to apoptosis in response to PARP1 inhibition.[22] We observed similar effects in BRCA knockdown HeLa cells treated with Olaparib or (±)-gossypol (Figure 4f); WB analysis, FACS (FACS = fluorescence-activated cell sorting), and caspase-3 enzymatic assays all indicated elevated apoptosis of drug-treated cells by caspase-3 activation.

To elucidate which isomeric form of (±)-gossypol bound to PARP1 BRCT, we carried out additional experiments with (−)-gossypol (Figure 5). ITC and FP results showed that (−)-gossypol/protein binding was marginally better ($K_d = 3.11 \pm 0.16$ μM by ITC; 0.47 ± 0.06 μM by FP; Figure 4b and S7) than that of the racemic mixture, and this preference was further supported by docking experiments (Figure 1c and S7a). (+)-Gossypol did not fit as snugly into the PARP1 BRCT domain as the (−)-isomer. (−)-Gossypol is a dialdehyde-

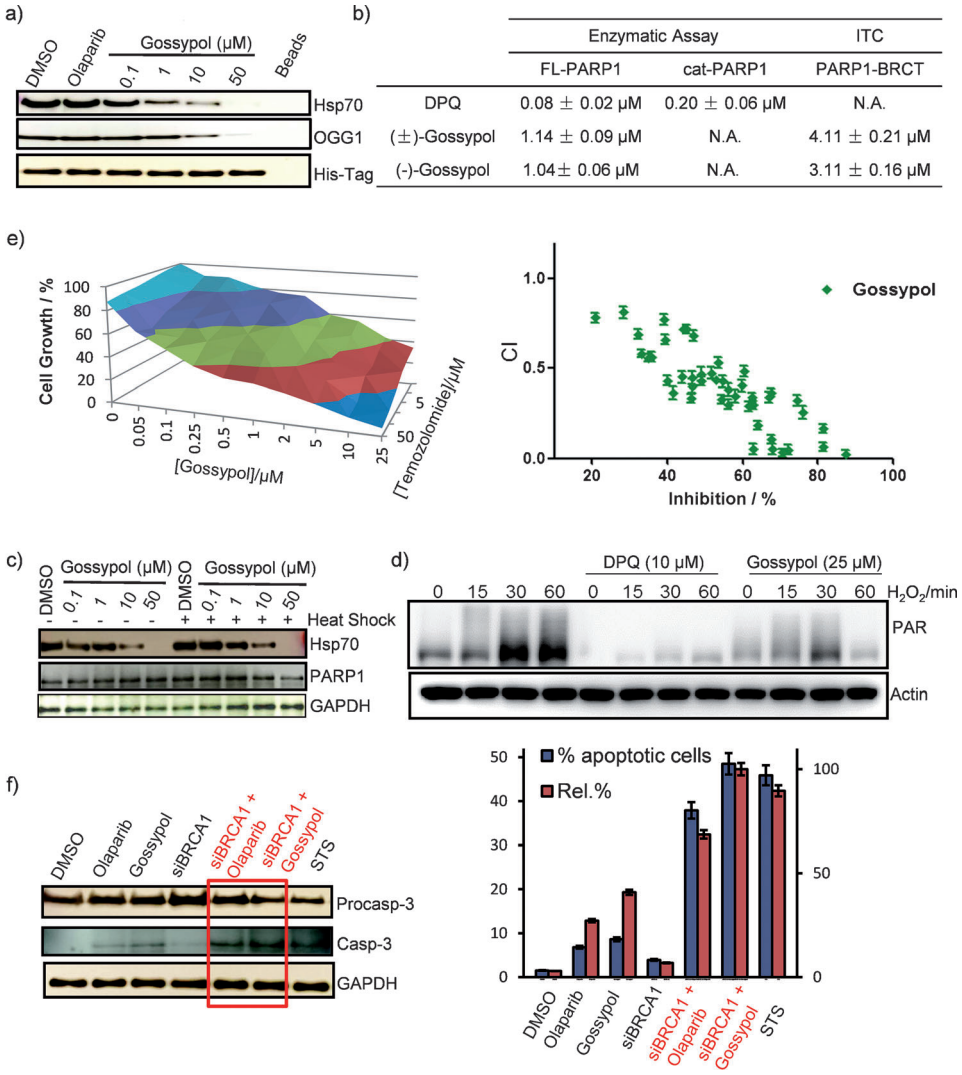

Figure 4. a) In vitro competitive binding of (±)-gossypol with cellular proteins for the PARP1 BRCT domain. HeLa cell lysates were used. b) (±)-Gossypol inhibits PARP1 activity by binding to its BRCT domain. c) In situ immunoprecipitation (IP) experiment showing that (±)-gossypol competed for PARP1 binding to Hsp70 in HeLa cells. d) (±)-Gossypol inhibited PAR formation in HeLa cells exposed to H_2O_2 (500 μM) for different periods of time. DPQ (10 μM) was used as a positive control. e) XTT anti-proliferation results of HeLa cells treated with different combinations of (±)-gossypol and Temozolomide. Right: Corresponding CI plot. f) Apoptosis analysis of BRCA1 knockdown HeLa cells treated with Olaparib or (±)-gossypol (25 μM each). Positive control: STS (staurosporine: 200 nM). Left: WB analysis. Right: FACS/caspase-3 enzymatic activity analysis of the same cells. % apoptotic cells determined by FACS using the Annexin-V kit. Rel%: Relative caspase-3 activity determined by Ac-DEVD-AMC. See the Supporting Information for details.

containing C_2-symmetric compound, and in ITC experiments, we consistently observed a 2:1 stoichiometry between the protein and (−)-gossypol (Figure S7b), which was further confirmed by chemical cross-linking experiments with $NaCNBH_3$. As shown in Figure 5b (left), upon incubation with 0.5 equivalents of (−)-gossypol, a significant amount of a PARP1 BRCT dimer was formed. Further evidence of (−)-gossypol-promoted dimerization of PARP1 was provided by successful cross-linking with both the full-length recombinant protein (Figure 5b; right) and endogenous PARP1 in living mammalian cells (Figure 5c). We found that this cross-linking reaction was both PARP1-BRCT- and (−)-gossypol-specific (Figure S10); neither the catalytic domain of PARP1 nor glutaldehyde (a general dialdehyde cross-linker) were capable of causing PARP1 dimerization. We thus suggest that as a novel small-molecule PPI inhibitor of PARP1, (−)-gossypol likely acts as a chemical dimerizer of the protein by promoting the reversible formation of covalently bound imines between its dialdehyde and the residue(s) in the PARP1-BRCT domain, leading to inhibition of the PARP1 enzymatic activity (Figure 5a). Attempts to solve the structure of the PARP1 BRCT/(±)-gossypol complex by X-ray crystallography and protein NMR spectroscopy failed owing to the low solubility of (±)-gossypol. We thus carried out site-directed mutagenesis, LC-MS/MS analysis, and docking experiments to identify potential residues that participated in the cross-linking. Docking results showed that Lys^{438}, Lys^{441}, and Lys^{447} in the PARP1 BRCT domain were near the (−)-gossypol binding site (Figure 1c). LC-MS/MS studies of the PARP1 BRCT/(−)-gossypol cross-linked product showed 92% sequence coverage, with the only missing peptide bearing Lys^{438} and Lys^{441} (Figure S12), indicating that either or both residues were modified by (−)-gossypol. Chemical cross-linking results showed that PARP1 BRCT dimer formation was abolished in the K438A and K441A mutants, but not with the K447A mutant (Figure 5d). We thus conclude that both Lys^{438} and Lys^{441} are key residues in PARP1, which directly participate in (−)-gossypol binding or in inducing dimerization. Finally, in a cellular thermal shift assay (CETSA),[23] PARP1 was efficiently stabilized by both Olaparib and (−)-gossypol in HeLa cells. This indicates that similar to Olaparib, (−)-gossypol was also engaged in physical binding to endogenous PARP1 in intake cells (Figure 5e).

In conclusion, we have successfully identified (±)-gossypol as the first cell-permeable small-molecule PPI inhibitor of PARP1. Further elucidation of its mechanism of action indicates that (−)-gossypol was mostly likely the isomer that

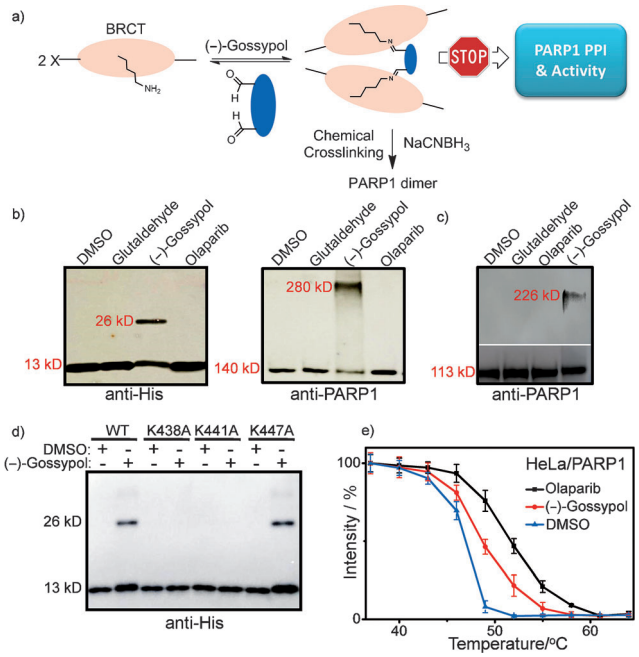

Figure 5. a) Proposed model of (−)-gossypol binding to the PARP1 BRCT domain, which leads to dimer formation, disruption of protein–protein interactions, and inhibition of PARP1 enzymatic activity. b) WB analysis of the recombinant $(His)_6$-tagged PARP1 BRCT/(−)-gossypol (2:1) complex formation (left) and the recombinant GST-fused full-length PARP1/(−)-gossypol (2:1) complex formation (right) upon reduction by $NaCNBH_3$. DMSO, glutaldehyde, and Olaparib were used as negative controls. c) WB analysis of chemically cross-linked PARP1 formation in live HeLa cells treated with (−)-gossypol. Longer exposure of the top portion of the gel was needed to detect the PARP1 dimer. d) WB analysis of chemically cross-linked bacterial lysates overexpressing the WT and mutant PARP1 BRCT domains (K438A, K441A, K447A) with (−)-gossypol. e) Graphical summary of the CETSA results of HeLa cells treated with Olaparib and (−)-gossypol (25 μM). See the Supporting Information for details.

bound to the PARP1 BRCT domain, causing the protein to dimerize and leading to subsequent inhibition of protein–protein interactions and the overall PARP1 enzymatic activity. Unlike previously reported PARP1 inhibitors, which target the enzyme's active site, the novel mechanism of (−)-gossypol should open new opportunities for the development of highly potent and specific PPI inhibitors against PARP1.

Received: November 2, 2014
Revised: December 7, 2014
Published online: January 7, 2015

Keywords: cancer · inhibitors · microarrays · PARP1 · protein–protein interactions

[1] B. A. Gilson, W. L. Kraus, *Nat. Rev. Mol. Cell Biol.* **2012**, *13*, 411 – 424.
[2] J. L. Riffell, C. J. Lord, A. Ashworth, *Nat. Rev. Drug Discovery* **2012**, *11*, 923 – 936.
[3] D. V. Ferraris, *J. Med. Chem.* **2010**, *53*, 4561 – 4584.
[4] E. Wahlberg, T. Karlberg, E. Kouznetsova, N. Markova, A. Macchiarulo, A. G. Thorsell, E. Pol, A. Frostell, T. Ekblad, D. Oncu, B. Kull, G. Michael, G. M. Robertson, R. Pellicciari, H. Schuler, J. Weigelt, *Nat. Biotechnol.* **2012**, *30*, 283 – 288.
[5] S. Vyas, P. Chang, *Nat. Rev. Cancer* **2014**, *14*, 502 – 509.
[6] M. F. Langelier, J. L. Planck, J. M. Pascal, *Science* **2012**, *336*, 728 – 732.
[7] C. C. Y. Leung, J. N. M. Glover, *Cell Cycle* **2011**, *10*, 2461 – 2470.
[8] P. A. Loeffler, M. J. Cuneo, G. A. Mueller, E. F. DeRose, S. A. Gabel, R. E. London, *BMC Struct. Biol.* **2011**, *11*, 37.
[9] a) J. T. Heale, A. R. Ball, J. A. Schmiesing, J.-S. Kim, X. Kong, S. Zhou, D. F. Hudson, W. C. Earnshaw, K. Yokomori, *Mol. Cell* **2006**, *21*, 837 – 848; b) P. Kotoglou, A. Kalaitzakis, P. Vezyraki, T. Tzavaras, L. Michalis, F. Dantzer, J. U. Jung, C. Angelidis, *Cell Stress Chaperones* **2009**, *14*, 391 – 406; c) H. N. Hooten, K. Kompaniez, J. Barnes, A. Lohani, M. K. Evans, *J. Biol. Chem.* **2011**, *286*, 44679 – 44690.
[10] M. O. Hottiger, P. O. Hassa, B. Luscher, H. Schuler, F. Koch-Nolte, *Trends Biochem. Sci.* **2009**, *35*, 208 – 219.
[11] a) H. Wu, J. Y. Ge, S. Q. Yao, *Angew. Chem. Int. Ed.* **2010**, *49*, 6528 – 6532; *Angew. Chem.* **2010**, *122*, 6678 – 6682; b) Z. Na, S. Pan, M. Uttamchandani, S. Q. Yao, *Angew. Chem. Int. Ed.* **2014**, *53*, 8421 – 8426; *Angew. Chem.* **2014**, *126*, 8561 – 8566.
[12] V. Azzarito, K. Long, N. S. Murphy, A. J. Wilson, *Nat. Chem.* **2013**, *5*, 161 – 173.
[13] M. R. Arkin, J. A. Wells, *Nat. Rev. Drug Discovery* **2004**, *3*, 301 – 317.
[14] a) K. Dodou, R. J. Anderson, D. A. Small, P. W. Groundwater, *Expert Opin. Invest. Drugs* **2005**, *14*, 1419 – 1434; b) G. Z. Tang, Z. Nikolovska-Coleska, S. Qiu, C.-Y. Yang, J. Guo, S. Wang, *J. Med. Chem.* **2008**, *51*, 717 – 720.
[15] H. C. Reinhardt, M. B. Yaffe, *Nat. Rev. Mol. Cell Biol.* **2013**, *14*, 563 – 580.
[16] C. H. S. Lu, H. Sun, F. B. A. Bakar, M. Uttamchandani, W. Zhou, Y.-C. Liou, S. Q. Yao, *Angew. Chem. Int. Ed.* **2008**, *47*, 7438 – 7441; *Angew. Chem.* **2008**, *120*, 7548 – 7551.
[17] M. Uttamchandani, E. W. S. Chan, G. Y. J. Chen, S. Q. Yao, *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2997 – 3000.
[18] C. Zhang, C. Y. J. Tan, J. Ge, Z. Na, G. Y. J. Chen, M. Uttamchandani, H. Sun, S. Q. Yao, *Angew. Chem. Int. Ed.* **2013**, *52*, 14060 – 14064; *Angew. Chem.* **2013**, *125*, 14310 – 14314.
[19] K. A. Menear, C. Adcock, R. Boulter, X. L. Cockcroft, L. Copsey, A. Cranston, K. J. Dillon, J. Drzewiecki, S. Garman, S. Gomez, H. Javaid, F. Kerrigan, C. Knights, A. Lau, V. M. Loh, I. T. W. Matthews, S. Moore, M. J. O'Connor, G. C. M. Smith, N. M. B. Martin, *J. Med. Chem.* **2008**, *51*, 6581 – 6591.
[20] J. Zhou, S. Ng, Q. Huang, Y. T. Wu, Z. Li, S. Q. Yao, H. M. Shen, *FEBS Lett.* **2013**, *587*, 170 – 177.
[21] Y. Shen, F. L. Rehman, Y. Feng, J. Boshuizen, I. Bajrami, R. Elliott, B. Wang, C. J. Lord, L. E. Post, A. Ashworth, *Clin. Cancer Res.* **2013**, *19*, 5003 – 5015.
[22] P. C. Fong, D. S. Boss, T. A. Yap, A. Tutt, P. Wu, M. Mergui-Roelvink, P. Mortimer, H. Swaisland, A. Lau, M. J. O'Connor, A. Ashworth, J. Carmichael, S. B. Kaye, J. H. M. Schellens, J. S. de Bono, *N. Engl. J. Med.* **2009**, *361*, 123 – 134.
[23] D. M. Molina, R. Jafari, M. Ignatushchenko, T. Seki, E. A. Larsson, C. Dan, L. Sreekumar, Y. Cao, P. Nordlund, *Science* **2013**, *341*, 84 – 87.