SUPPLEMENTARY MATERIAL

Targeting BRCA1- and BRCA2-deficient cells with RAD52 small molecule inhibitors

F. Huang[1], N. Goyal[1], K. Sullivan[2], K. Hanamshet[1], M. Patel[1], O. M. Mazin[1], Charles X. Wang[1], W. Frank An[3], J. Spoonamore[3], Shailesh Metkar[3], K. A. Emmitte[4], S. Cocklin[1], T. Skorski[2], and A. V. Mazin[1*].

[1]Department of Biochemistry and Molecular Biology, Drexel University College of Medicine, Philadelphia, PA 19102, USA; [2]Department of Microbiology and Immunology, and Fels Institute for Cancer Research and Molecular Biology, Temple University School of Medicine, Philadelphia, PA 10140, USA; [3]Broad Institute of Harvard and Massachusetts Institute of Technology, Cambridge, Massachusetts 02142; [4]Vanderbilt Specialized Chemistry Center for Accelerated Probe Development, Vanderbilt Center for Neuroscience Drug Discovery, Department of Pharmacology and Chemistry, Vanderbilt University, Nashville, TN 37232, USA

*Corresponding Author:
Alexander Mazin, Ph. D.
Drexel University College of Medicine
Department of Biochemistry and Molecular Biology
245 N 15th Street, MS 497, NCB, Room 10103
Philadelphia, PA 19102–1192
Tel: 215–762–7195; Fax: 215–762–4452
E-mail: amazin@drexelmed.edu

Content:

Supplementary Figure 1. Analysis of RAD51 and RAD52 proteins in a SDS-polyacrylamide gel. Proteins were stained with Coomassie blue. Lane M, Migration markers; Lane 1, Rad52; Lane 2, RAD51. 1μg of each protein was loaded on a 12% SDS-polyacrylamide gel.

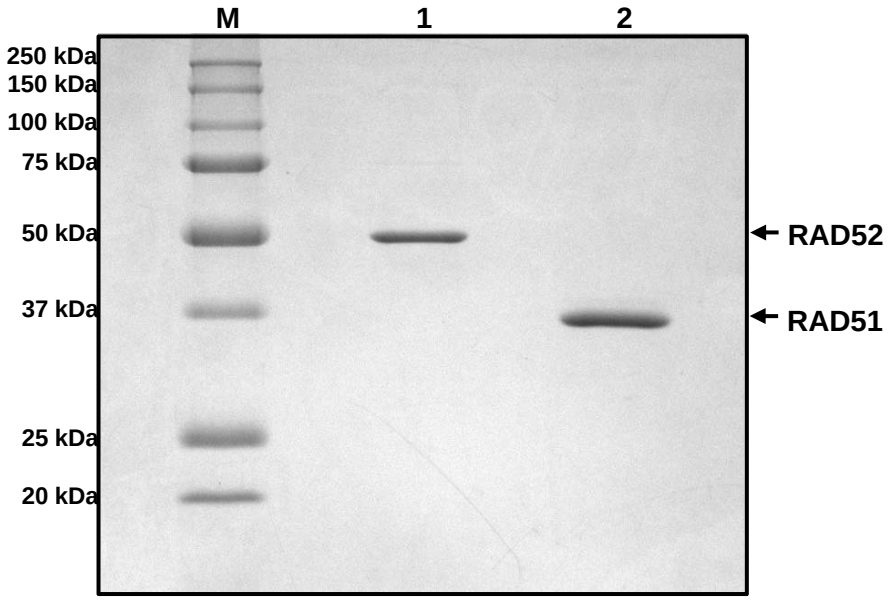
Supplementary Figure 2. The effect of D-I03 concentration on the initial rate of ssDNA annealing promoted by RAD52. The rates of RAD52-promoted ssDNA annealing were calculated based on the data in Fig. 1C. The data are the mean of 3 independent measurements; error bars represent the SD.

Supplementary Figure 3. Structures of RAD52 inhibitors.

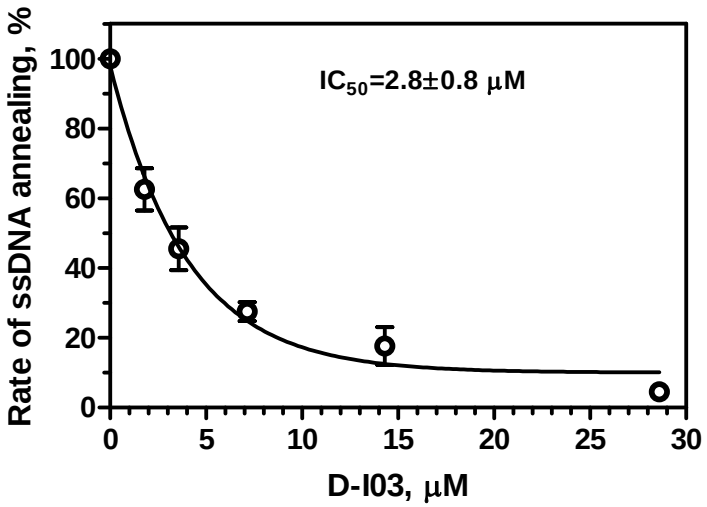
Supplementary Figure 4. The effect of the RAD52 inhibitors on DNA pairing activity of RAD51 in the D-loop assay. To form RAD51 nucleoprotein filament, RAD51 (1 μM) was incubated with ^{32}P-labeled ssDNA (3 μM, nt) in buffer containing 25 mM Tris-Acetate, pH 7.5, 100 $\mu g \cdot ml^{-1}$ BSA, 1 mM calcium chloride, 1 mM ATP and 2 mM DTT for 15 min at 37 °C. Then inhibitors were added at the concentrations that correspond to their 10 x IC_{50} values for RAD52 pairing activity (see Figure 2C; Supplementary Table 2) and incubation continued for 15 min at 37 °C. D-loop formation was initiated by addition of supercoiled pUC19 DNA (50 μM, nucleotides) and was carried out 15 min at 37 °C. The reactions were stopped and the products processed as described in Materials and Methods. Error bars indicate SD.

Supplementary Figure 5. The effect of D-I03 on the repair of the I-SceI-induced DSBs in U2OS cells carrying the SSA-GFP reporter was determined by flow cytometry. Green fluorescence (GRN-Hlog) was plotted against red fluorescence (RED-Hlog) for the sample of 10,000 cells. The GFP+ population is denoted by the elliptical M1 marker. Cells with I-SceI-induced DSBs were treated with D-I03 at 0, 5, 10, 15, 20, or 30 μM (panels 1−6, respectively). In a negative control, U2OS cells were transfected with pUC19, instead of pCBASce that expresses I-SceI (panel 7). U2OS cells transfected with pMX-GFP plasmid expressing GFP protein were used as a positive control (panel 8).

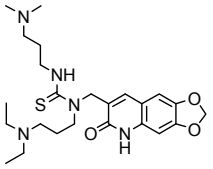
Supplementary Figure 6. The effect of D-I03 on the repair of the I-SceI-induced DSBs in U2OS cells carrying the HDR-GFP reporter (to measure gene conversion) was determined by flow cytometry. Green fluorescence (GRN-Hlog) was plotted against red fluorescence (RED-Hlog) for the sample of 10,000 cells. The GFP+ population is denoted by the elliptical M1 marker. Cells with I-SceI-induced DSBs were treated with D-I03 at 0, 5, 10, 15, 20, or 30 μM (panels 1−6, respectively). In a negative control, U2OS cells were transfected with pUC19, instead of pCBASce (panel 7). U2OS cells transfected with pMX-GFP plasmid expressing GFP protein were used as a positive control (panel 8).



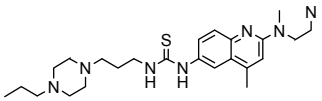
Supplementary Fig. 1



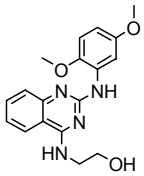
Supplementary Fig. 2



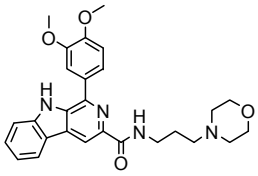
D-A13



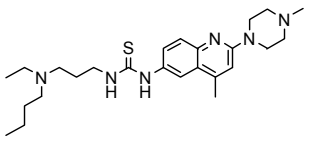
D-E05



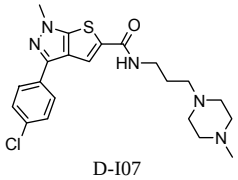
D-I05



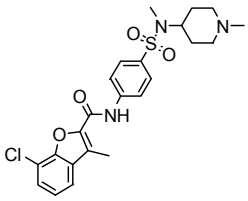
D-A19



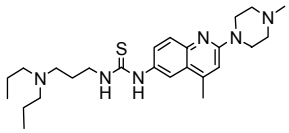
D-G09



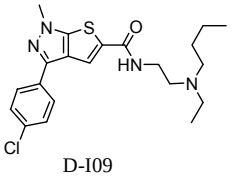
D-I07



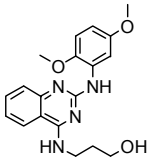
D-A21



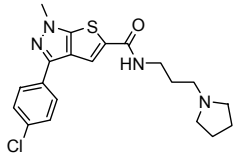
D-G11



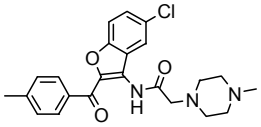
D-I09



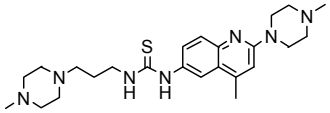
D-G23



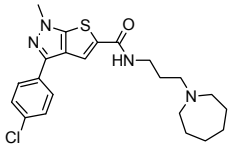
D-I19



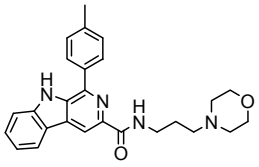
D-C17



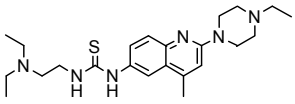
D-I01



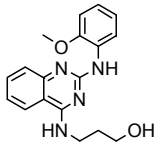
D-I11



D-C19

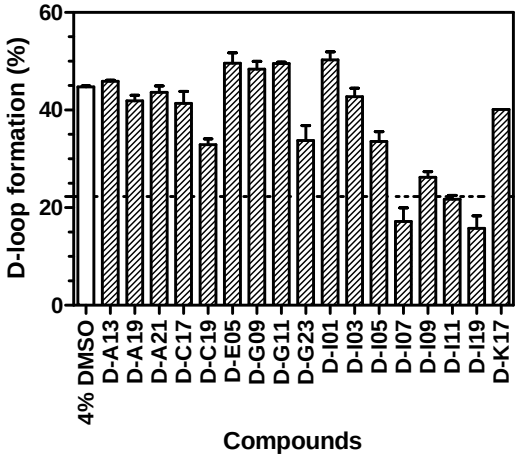


D-I03

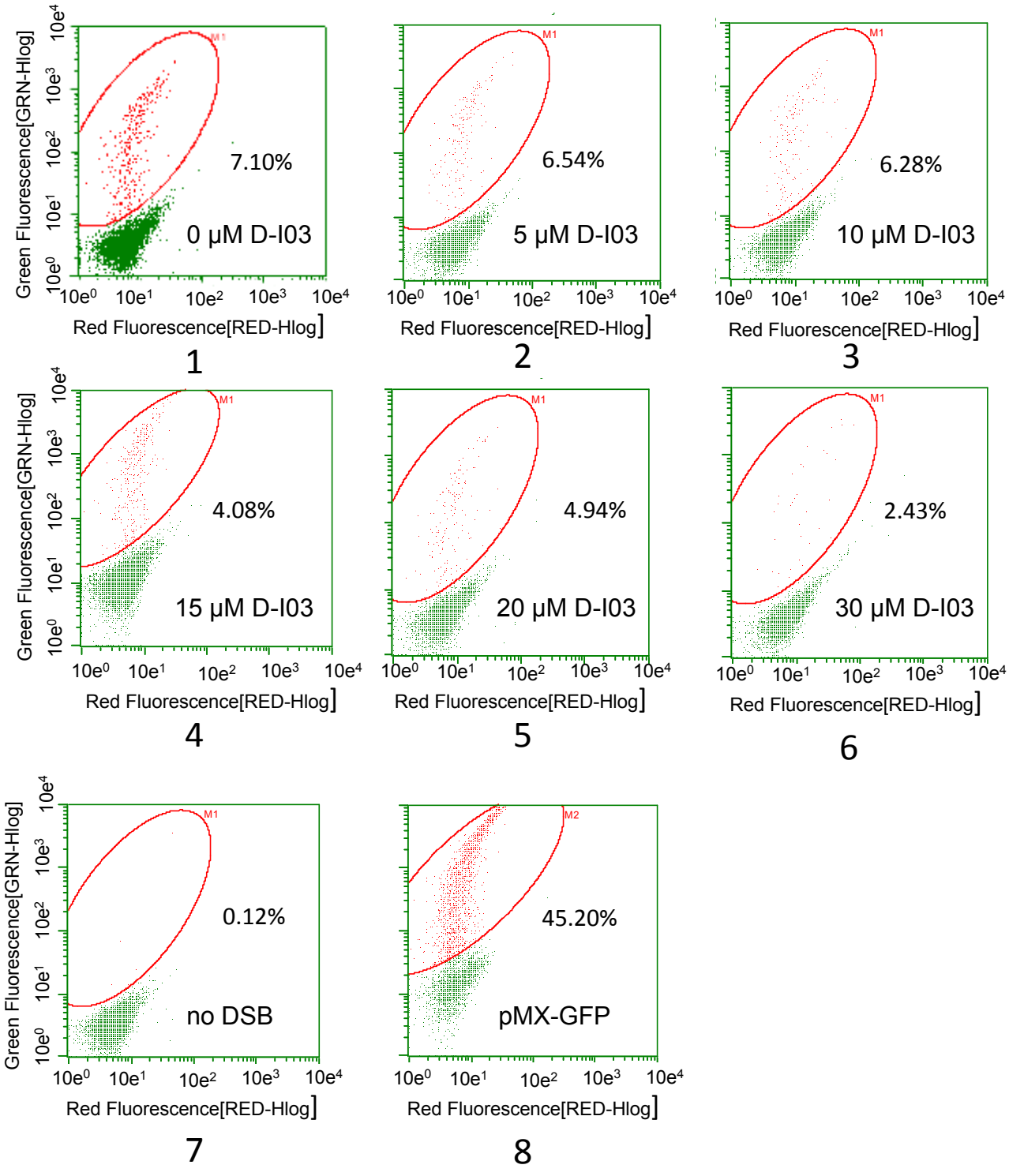


D-K17

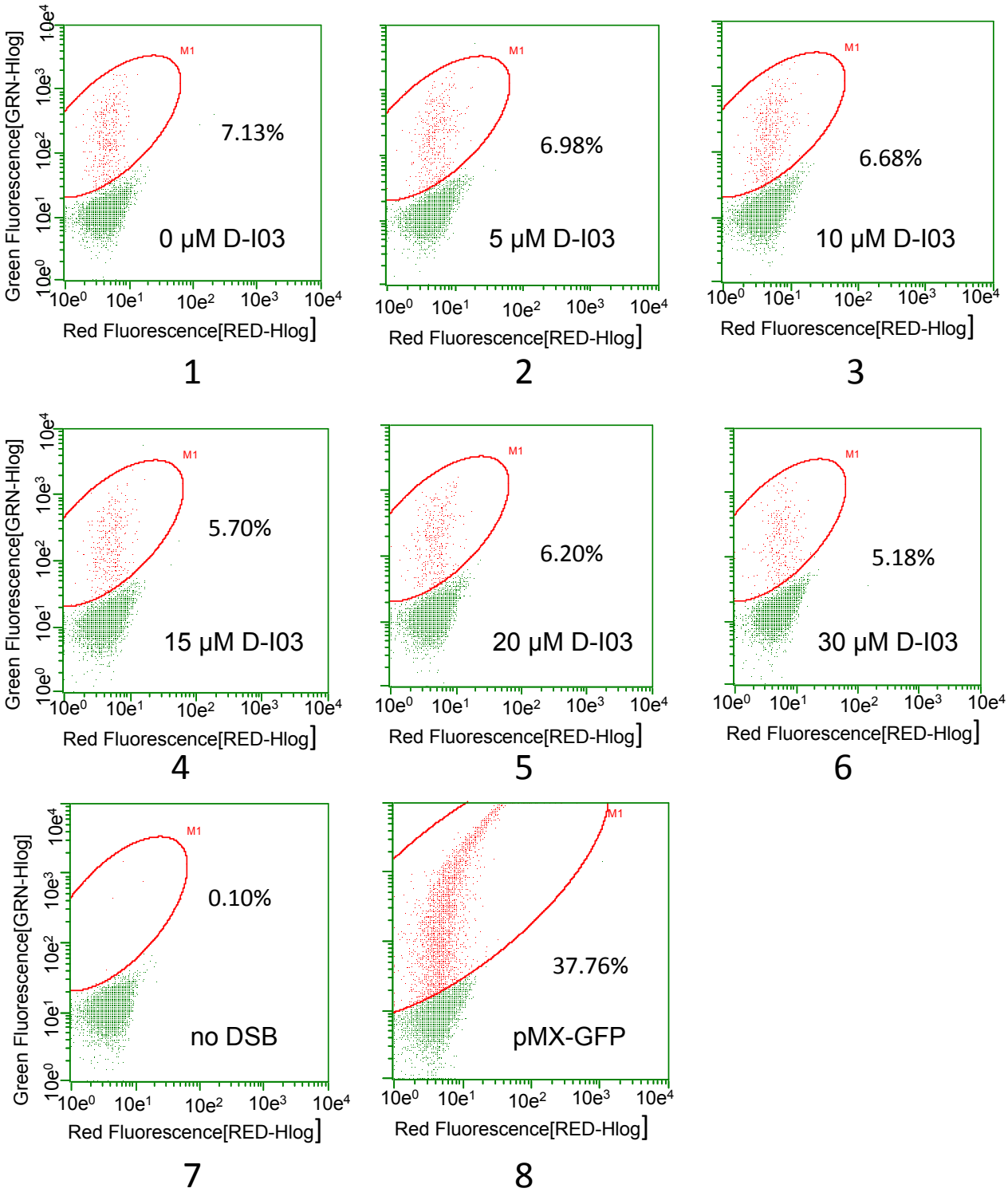
Supplementary Fig. 3



Supplementary Fig. 4



Supplementary Fig. 5



Supplementary Fig. 6

Supplementary Table 1. Sequences of the oligonucleotides

N	Length, nt	Sequence (5'→3')
337-FLU	60	FLU-CACTGTGATGCACGATGATCGACGACAGTAGTCAGT GCTGGGTCAACATCTGTATGCAGG
1337-BHQ1	39	AGCACTGACTACTGTCGTCGATCATCGTGCATCACAGTG–BHQ1
265-55	55	ATACAGATGTTGACCCAGCACTGACTACTGTCGTCAATCAT CGTGCATCACAGTG
90	90	CGGGTGTCGGGGCTGGCTTAACTATGCGGCATCAGAGCA GATTGTACT GAG AGT GCA CCA TAT GCG GTG TGA AAT ACC GCA CAG ATG CGT

Note: "FLU" and "BHQ1" denote Fluorescein and Black Hole Quencher 1, respectively

Supplementary Table 2. **Effect of inhibitors on the ssDNA annealing and DNA pairing activities of RAD52 and on the DNA pairing activity of RAD51**

Inhibitors	ssDNA annealing (Fluorescence quenching), IC_{50}, μM	Inhibition of DNA pairing (D-loop formation), IC_{50}, μM	Inhibition of RAD51 paring (D-loop formation), %*
D-A13	5.2	13.6±0.64	102.5±1.1
D-A19	4.8	7.2±0.28	93.7±2.1
D-A21	9.8	16.2±0.42	97.5±2.5
D-C17	6.0	17.6±0.78	92.5±5.0
D-C19	2.0	4.3±0.35	73.6± 2.2
D-E05	1.7	11.3±0.35	110.9±4.3
D-G09	2.0	14.8±2.47	108.2±3.1
D-G11	6.0	8.9±1.6	110.7±0.2
D-G23	5.6	7.2±0.96	75.6±7.1
D-I01	3.6	15.4±0.57	112.5±4.2
D-I03	5.0	8.0±1.7	95.5±4.3
D-I05	4.3	8.8±0.42	75.1±4.8
D-I07	2.0	2.7±0.35	38.4±6.4
D-I09	6.8	10.6±1.4	58.6±2.9
D-I11	4.1	6.7±0.78	48.6±2.0
D-I19	3.5	4.1±0.14	35.3±5.8
D-K17	2.9	4.8±0.85	89.7±0.4

Note:* The effect of the inhibitors on RAD51 DNA pairing activity was measured at the concentrations that correspond their 10 x IC_{50} for RAD52 pairing activity. 100% of the D-loop yield correspond to the extent of reaction in the presence of 4% DMSO; the actual extent of D-loops was 44.7±0.2 % under these conditions.

Supplementary Table 3. Calculated properties of D-I03 and D-G23 compounds*

	D-I03	D-G23
molecular weight	429	354
cLogP	3.65	3.29
TPSA	47	89
HBD	2	3
HBA	5	7
rotatable bonds	7	7

* calculated using ADRIANA.code

TPSA = topological polar surface area
HBD = hydrogen bond donors
HBA = hydrogen bond acceptors

(https://www.molecular-networks.com/files/docs/adrianacode/adrianacode_flyer.pdf)