Small-molecule Inhibitors of Bacterial AddAB and RecBCD Helicase-nuclease DNA Repair Enzymes

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Supporting information

TABLE S1

Summary of protocol for screen for AddAB or RecBCD inhibitors in 1536-well plate format

Step	Operation	Condition	Comments
1	Medium dispensing	3 μL/well	Medium is Cation Adjusted Mueller Hinton Broth (CAMHB)
2	Compound addition	60 nL/well	Test concn, 12 μM; DMSO concn, 1.2%
3	Cell dispensing	1 μL/well	Bacteria grown in CAMHB to 2.5 x 10^7 cfu/ml
4	Incubation	60 min at 37 °C	
5	Phage addition	1 uL/well	Phage concn, 5 x 10^5/ml; MOI, 0.02
6	Incubation	18 hr at 37 °C	
7	Optical density determination	Read plate	OD_{600} optimized absorbance read on PerkinElmer Envision

TABLE S2

uHTS campaign summary and results

Step	Screen type	Target	Number of compounds tested	Selection criteria	Number of selected compounds	PubChem AID[e]	Assay statistics	
							Z'	S/B
1	Primary screen	AddAB	326,100	Inhibition >12.16%[a]	937	435030	0.91±0.02	3.60±0.15
2	Confirmation	AddAB	885	Inhibition >12.16%[b]	256	488942	0.84±0.02	2.55±0.07
3	Counter-screen	RecBCD	885	Inhibition >3.95%[c]	NA[f]	488955	0.93±0.02	3.73±0.32
3b	Counter-screen	*E. coli* V3065	885	Inhibition >24.60%[d]	NA[f]	488956	0.88±0.31	2.58±0.09
4a	Titration	AddAB	225	IC_{50} <10 μM for AddAB and >10 μM for RecBCD and *E. coli* V3065	7	492959	0.91±0.01	2.61±0.06
4b	Selectivity	RecBCD				492958	0.93±0.01	3.46±0.07
4c	Cytotoxicity	*E. coli* V3065				492957	0.89±0.02	2.58±0.06

[a] The primary screen hit-cutoff was calculated at the average percent inhibition of all test compounds plus three times the standard deviation.
[b] The hit-cutoff calculated for the primary run was also applied to the confirmation run.
[c] The hit-cutoff calculated for the counterscreen was derived from the average percent inhibition of all DMSO-only wells tested plus three times the standard deviation.
[d] The hit-cutoff calculated for the counterscreen was derived from the average percent inhibition of all DMSO-only wells tested plus eight times the standard deviation.
[e] PubChem AIDs are accessible on-line at http://www.ncbi.nlm.nih.gov/sites/entrez?db=pcassay&term=xxxxxx, where xxxxxx represents the PubChem AID number in the table
[f] NA, not applicable.

TABLE S3
***E. coli* strains**

Strain	Genotype	Ref.
V66	*hisG4 argA21 met recF143 rpsL31 galK2 xyl-5* F^- λ^-	(1)
V67	*recB21*::IS*186 hisG4 argA21 met recF143 rpsL31 galK2 xyl-5* F^- λ^-	(1)
V1306	*thi-1 relA1* λ^- (Hfr PO44)	(1)
V2831	*ΔrecBCD2731 <kan> hisG4 met recF143 rpsL31 galK2 xyl-5* F^- λ^-	(2)
V3060	*ΔrecBCD2731 <kan> hisG4 met recF143 rpsL31 galK2 xyl-5* F^- (λ DE3)	(3)
V3065	*ΔrecBCD2731 <kan> hisG4 met recF143 rpsL31 galK2 xyl-5* F^- λ^- (pSA405)	
V3069	*ΔrecBCD2731 <kan> hisG4 met recF143 rpsL31 galK2 xyl-5* F^- λ^- (pETDuet-1)	
JC8679	*thr-1 leuB6 ara-14 proA2 lacY1 tsx-33 galK2 hisG4 rpsL31 xyl-5 mtl-1 argE3 thi-1 recB21 recC22 sbcA23 supE44* F^- λ^-	(4)
JC9387	As JC8679 but *sbcA*$^+$ *sbcB15 sbcC* (*D*) *sup*$^+$ F^- λ^-	(4)
594	*lac-3350 galK2 galT22 rpsL179* F^- λ^-	(5)
C600	*thr-1 leuB6 thi-1 lacY1 tonA21 supE44 rfbD1* F^- λ^-	(6)

TABLE S4
Phage λ strains

Strain	Genotype[a]	Source[a] or ref.
1081	*susJ6 b1453 cI857* χ^+*D123*	(1, 7)
1082	*b1453* χ^+*D123 susR5*	(1, 7)
1083	*susJ6 b1453* χ^+*76 cI857*	(1, 7)
1084	*b1453* χ^+*76 susR5*	(1, 7)
DE3	*imm21 Δnin5 Sam7* P_{lacUV5} *gene 1* (T7 RNA polymerase)	Novagen

[a] *b1453* is a deletion removing *red*, which encodes recombination proteins exo and beta, and *gam*, which encodes an inhibitor of RecBCD. These phage recombine by the *E. coli* RecBCD pathway.

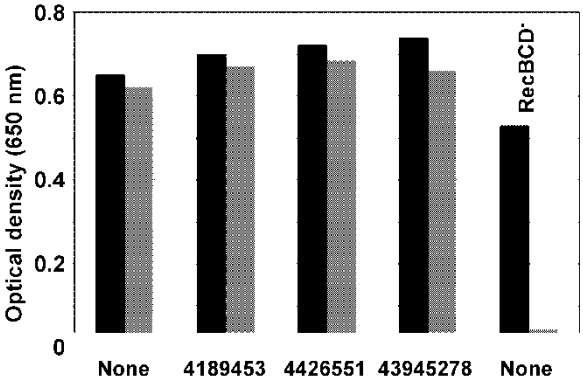
TABLE S5
Plasmids

Plasmid	Vector and insertion site	*E. coli* or *H. pylori* insert	Source or ref.
pBR322		None	(8)
pETDuet-1		None	Novagen
pACYCDuet-1		None	Novagen
pSA21	pBR322, *Bam*HI	*E. coli recB*^{21}*CD*	(9)
pMR3	pBR322, *Bam*HI	*E. coli recBCD* wild type	(2)
pSA405	pETDuet-1, *Nde*I *Pst*I	*H. pylori addAB* wild type	(3)
pSA502	pACYCDuet-1, *Nco*I *Pst*I	*H. pylori recA* wild type	(3)
pSA520	pBR322, *Hin*dIII	T4 *gene 2 amN51* (W247*UAG)	This work
pSA524	pBR322, *Hin*dIII	T4 *gene 2 am149* (W247*UAG, A248*UAG, N249*UAA)	This work
pSA600	pBR322	None; bp 381 – 1624 deleted	This work
pSA607	pSA600, *Eco*RI *Bam*HI	*E. coli recBD* at *Bam*HI, *recC* at *Eco*RI	This work

TABLE S6
Oligonucleotides

Oligonucleotide number	Nucleotide sequence
OL2636	5’ CATATGAAGCTTGTCAGTGTTTGCTGCAAATACTCCCCATG 3’
OL2637	5’ CATATGAAGCTTCACCGTTCTCATTCACATGATATAC 3’
OL2652	5’ TGAAATCGCCCCGAAAGACTAGTAGTAAGTTGTGTTGATGCCACTTCAGC 3’
OL2653	5’ GCTGAAGTGGCATCAACACAACTTACTACTAGTCTTTCGGGGCGATTTCA 3’

TABLE S7
Activity of iminobenzothiazoles in T4 *gene 2* mutant screen and Hfr crosses



Compound (100 μM)	Relative Hfr recombinant frequency [b]
None	1.0
CID 4189453	0.64, 0.72
CID 4426551	0.78, 0.67
CID 43945278	0.57, 0.47

Left. Growth of strain V66 in the absence (black bars) or presence (gray bars) of phage T4 *gene 2* triple nonsense mutant, as in Fig. S1, in the presence of the indicated compounds (100 μM). “RecBCD$^-$” used strain V67 (*recB21*). Data are the mean of two wells; range is <5% of mean.

Right. The frequency of His$^+$ Str^R recombinants in matings between strains V66 (F$^-$ *recBCD*$^+$ *hisG4 rpsL31*) and V1306 (Hfr PO44 *hisG*$^+$ *rpsL*$^+$) in the presence of compound is expressed as a fraction of that in the absence of compound (7.6 and 8.4 % per viable Hfr cell in the two experiments for which data are shown). Structures are in Fig. 2.

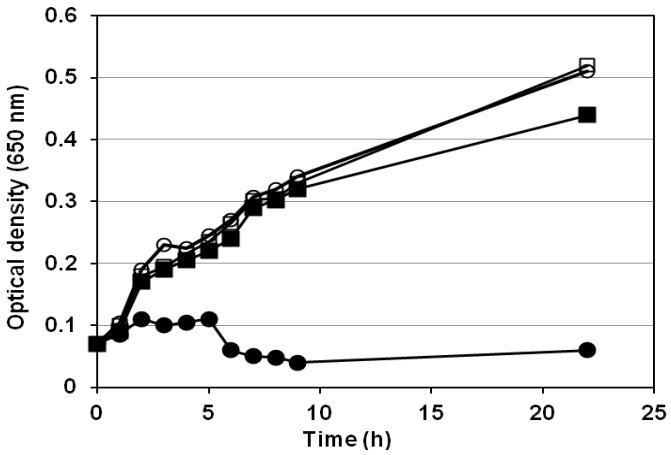


Figure S1. **T4 *gene 2* mutant phage prevent the growth of *E. coli* lacking RecBCD enzyme but not growth of wild type**. *E. coli* strain V66 (*recBCD*$^+$; open symbols) and strain V67 (*recB21*; closed symbols) were infected with T4 *gene 2* triple non-nonsense mutant phage (MOI = 0.01; circles) or not (squares). Cultures were 0.1 ml in a 96-well plate, which was shaken at 37 °C in an incubated plate reader. Each data point is the mean of 24 wells; SEM is within the size of the symbols. Similar results were found with *E. coli* expressing *H. pylori* AddAB.

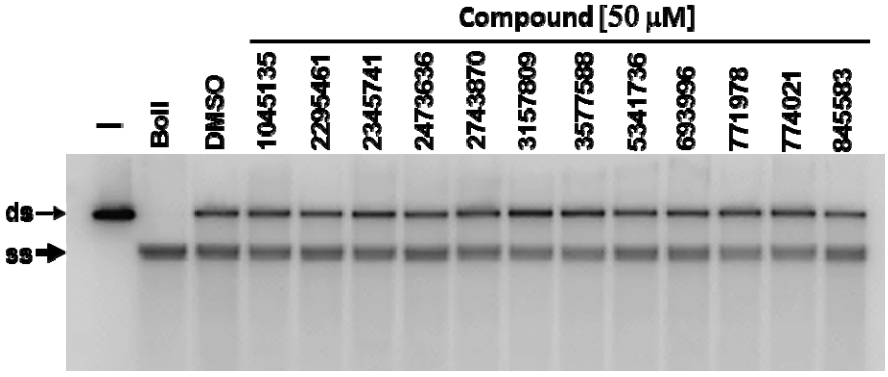


Figure S2. **AddAB DNA unwinding activity is not altered by compounds.** DNA unwinding by AddAB enzyme was assayed in the presence of compound (50 µM). Unwinding is indicated by the amount of ss DNA (heavy arrow). Structures are in Fig. 2.

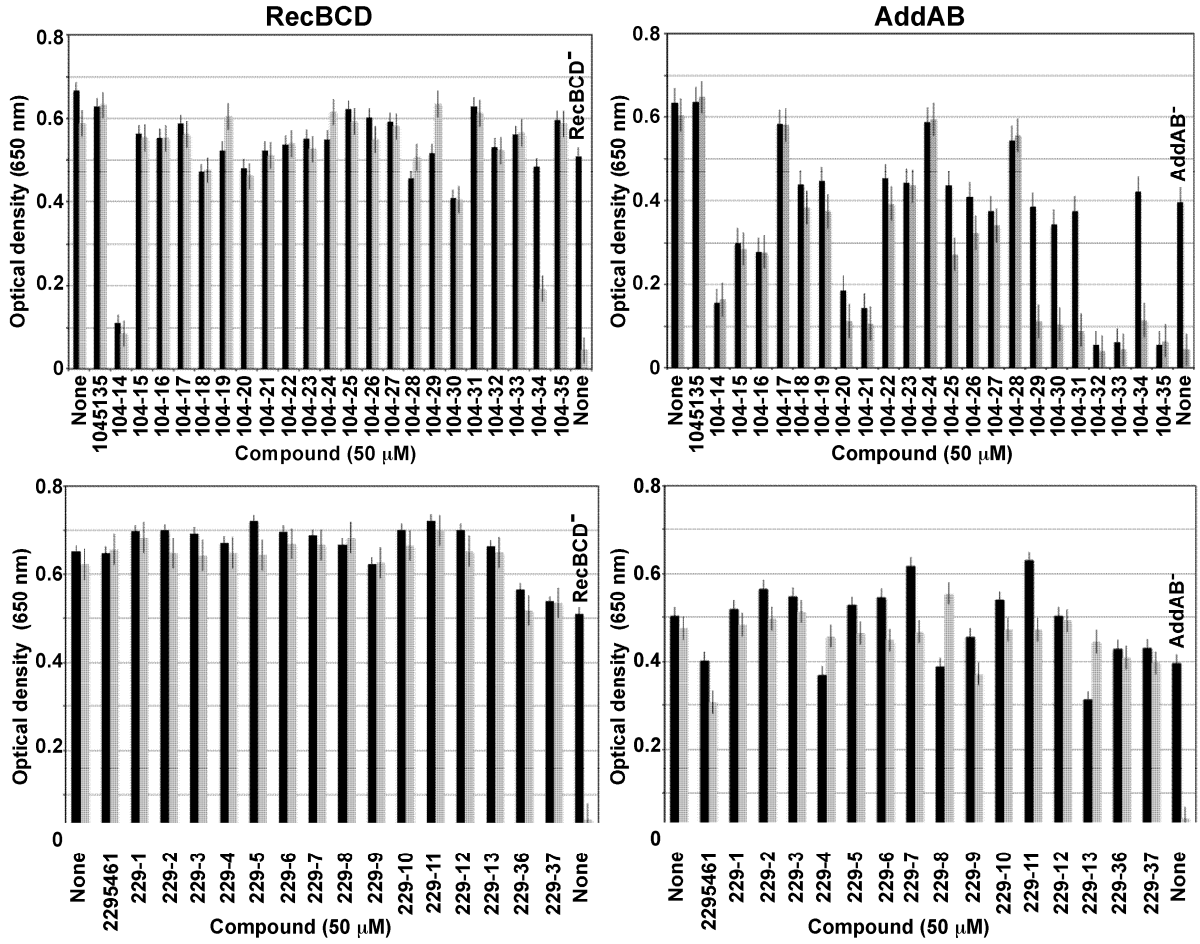


Figure S3. **Screen for active derivatives of CID 1045135 and CID 2295461.** Compounds were screened in 96-well format. Strain V66 (*recBCD*$^+$) or strain V67 (*recB21*; RecBCD$^-$) (*left panels*) or strain V3065 (*addAB*$^+$) or strain V3069 (empty vector control; AddAB$^-$) (*right panels*) in the presence of the indicated compound (50 μM) were infected with T4 *gene 2* triple nonsense mutant (grey bar; MOI = 0.01) or not (black bar), and the optical density measured after ~20 h of incubation. Data are the mean and SEM of 4 wells in 2 independent experiments. Structures and CID numbers are in Figs. 2, S4, and S6.

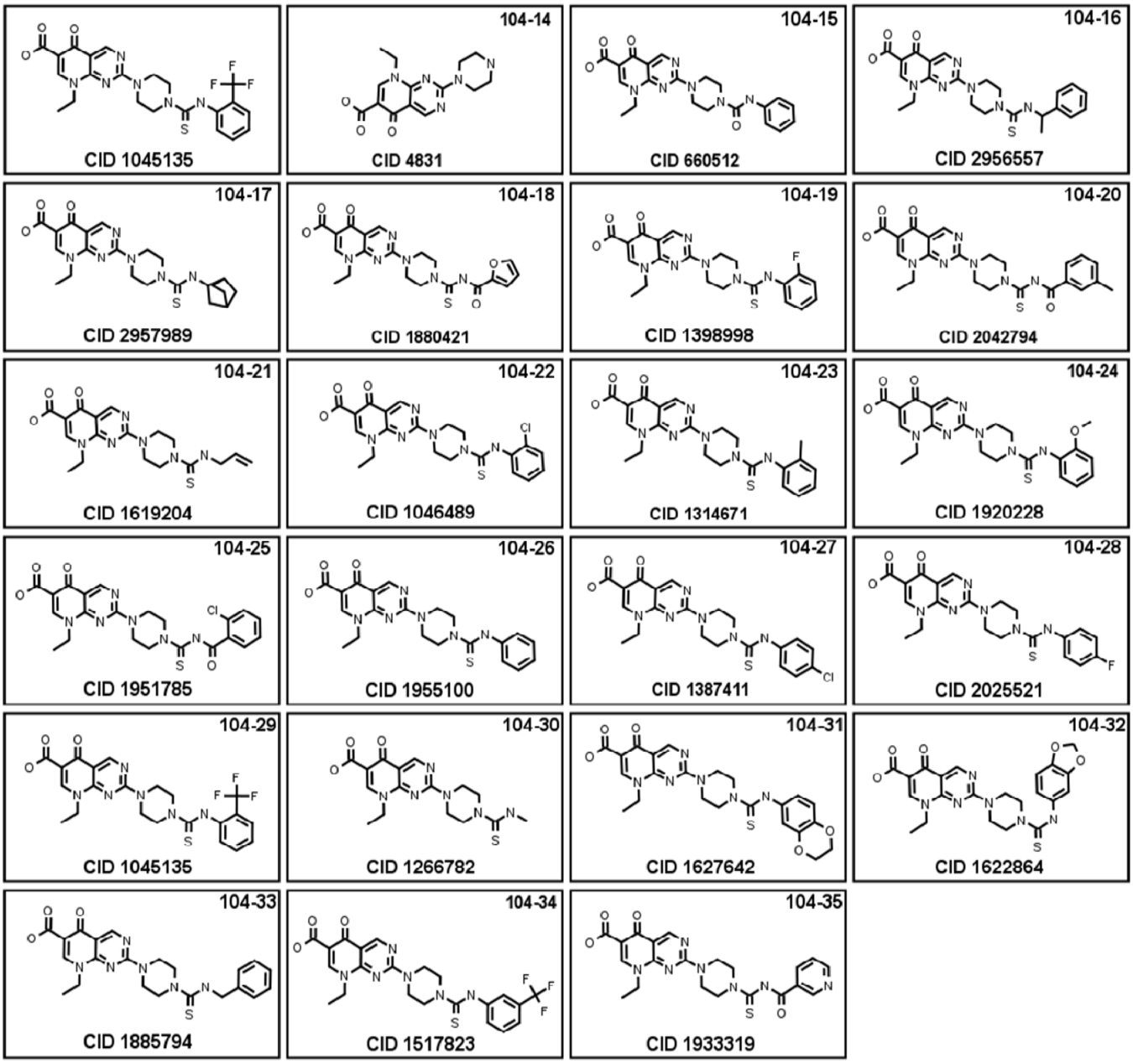


Figure S4. **Structures of compounds related to CID 1045135.** Trivial names, such 104-16 in upper right corner of each panel, are also used in the text and supplemental information.

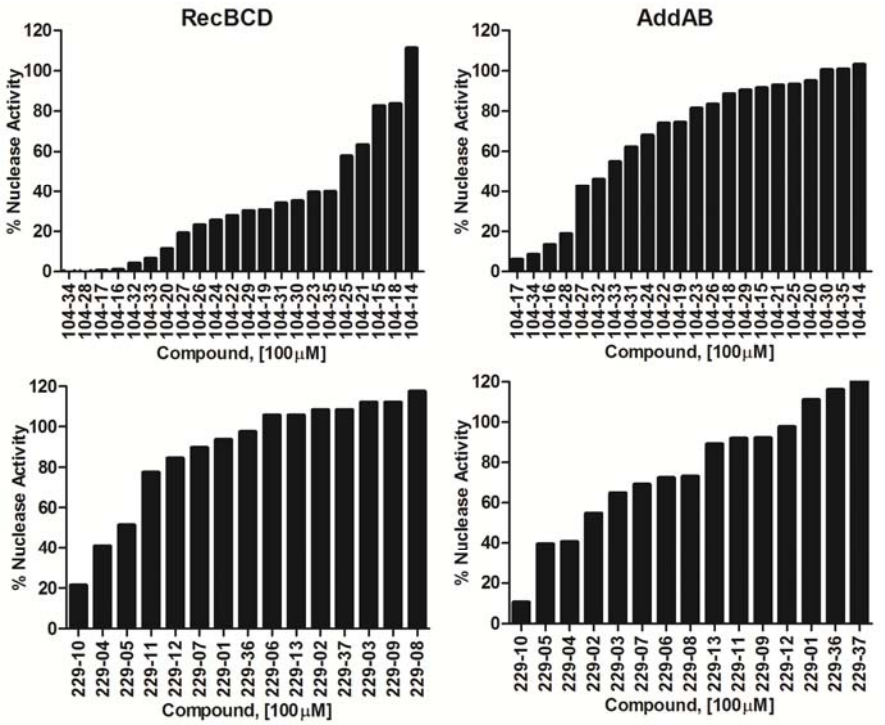


Figure S5. **Inhibition of AddAB and RecBCD nuclease activities by derivatives of CID 1045135 and CID 2295461.** ds exonuclease activity was measured in the presence of the indicated concentration of each compound and expressed as a percent of the activity in the absence of compound. A separate experiment with RecBCD and 50 μM CID 1045135 derivatives showed the same pattern. Structures and CID numbers are in Figs. 2, S4, and S6.

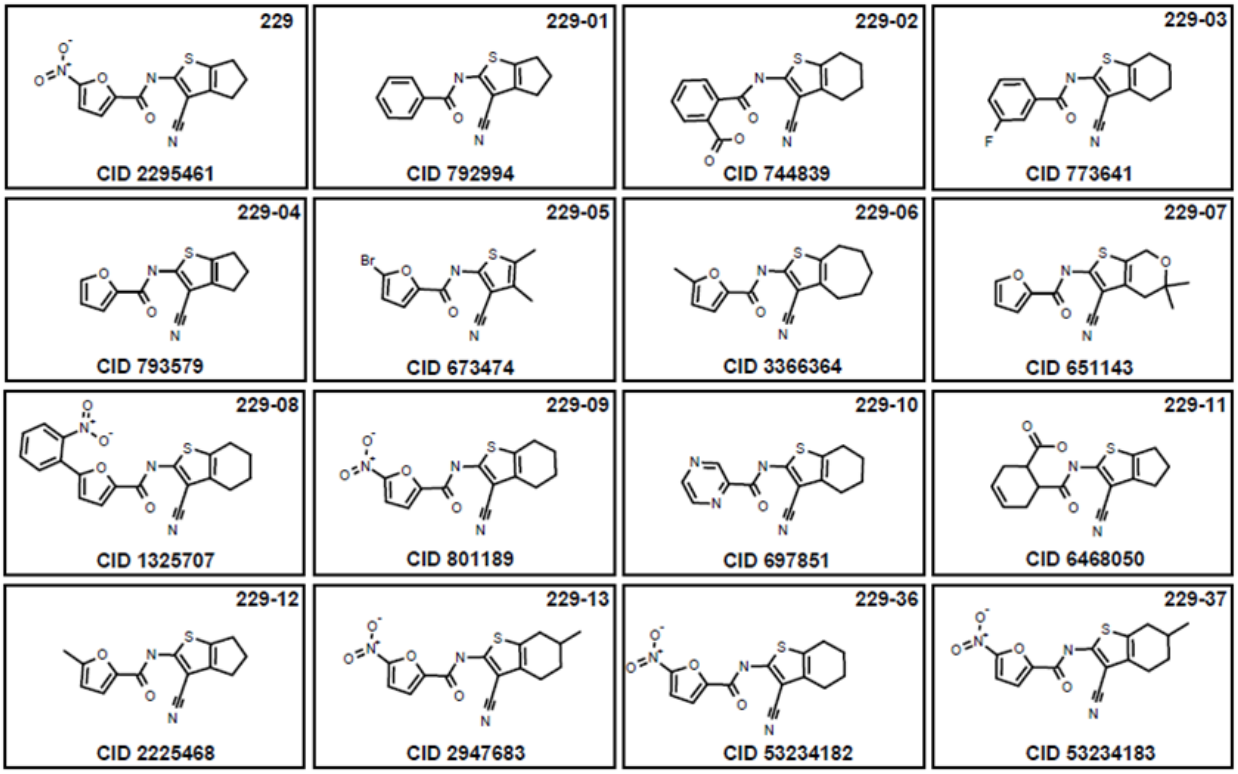


Figure S6. **Structures of compounds related to CID 2295461.** Trivial names, such 229-01 in upper right corner of each panel, are also used in the text and supplemental information.

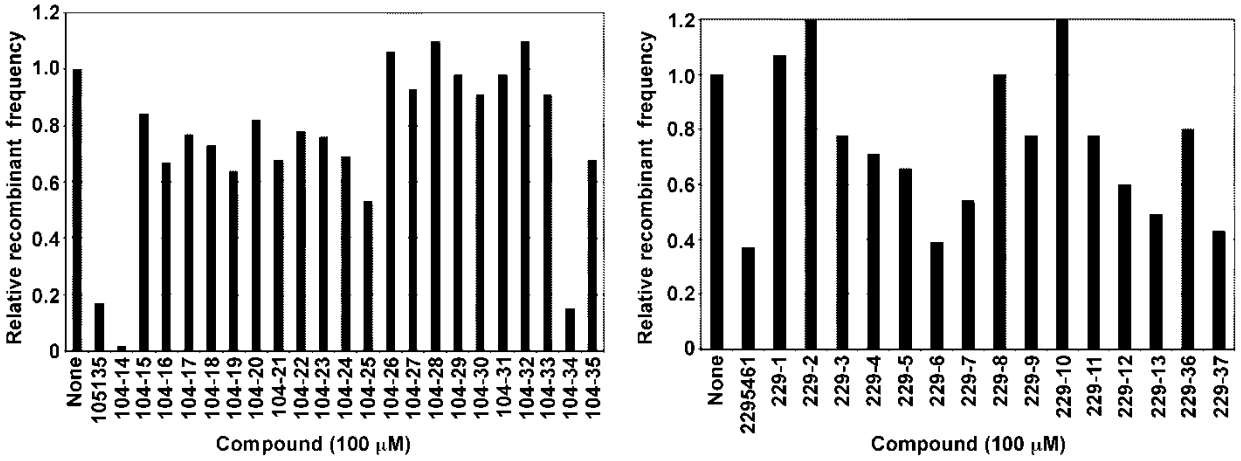


Figure S7. **Inhibition of *E. coli* Hfr recombination by derivatives of CID 1045135 and CID 2295461.** The frequency of His^+ Str^R recombinants in matings between strains V66 (F^- *recBCD*$^+$ *hisG4 rpsL31*) and V1306 (Hfr PO44 *hisG*$^+$ *rpsL*$^+$) in the presence of compound is expressed as a fraction of that in the absence of compound (4.4 % per viable Hfr cell). Structures and CID numbers are in Figs. 2, S4, and S6.

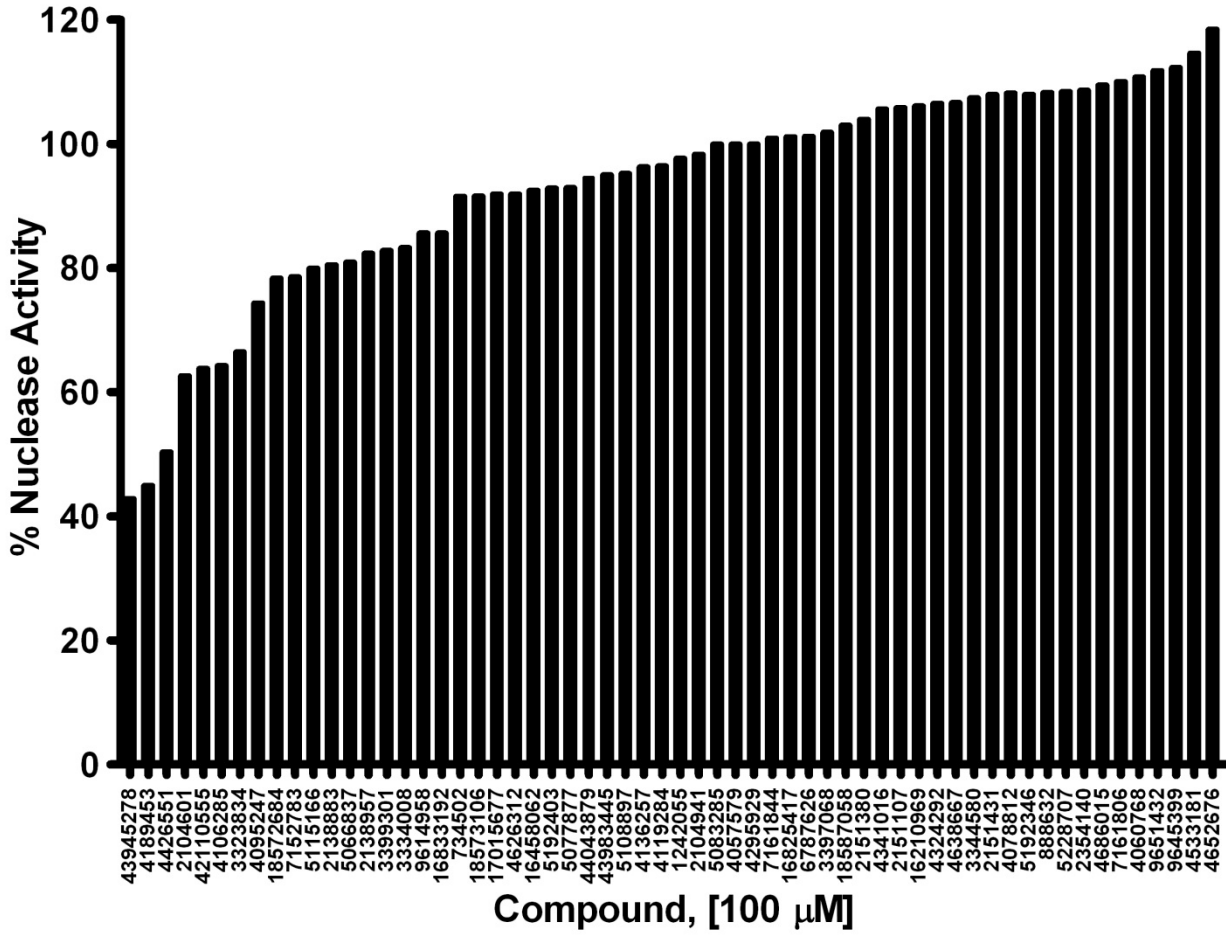


Figure S8. **Inhibition of RecBCD nuclease activity by "iminobenzothiazoles."** ds exonuclease activity was measured in the presence of each compound (100 μM; CID numbers indicated) and expressed as a percent of the activity in the absence of compound. Structures of the three most active compounds are in Fig. 2.

References for Supporting Information

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