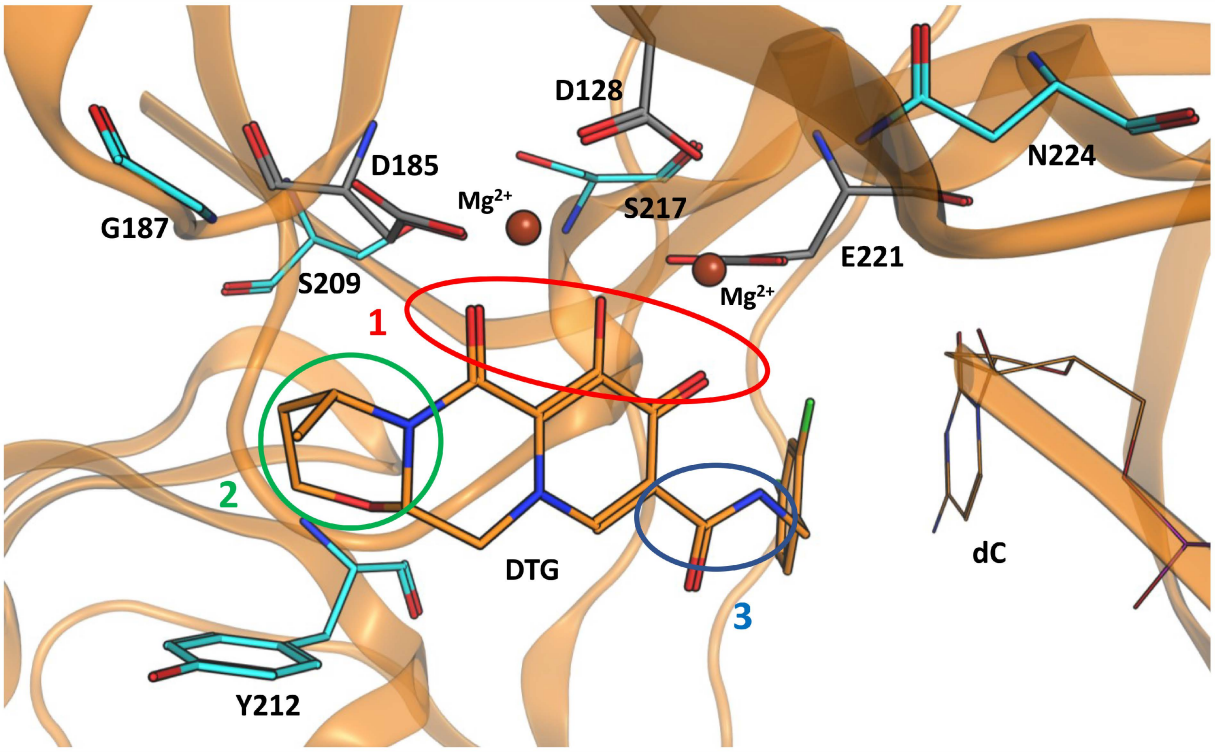

Supplementary Figure 1. DTG bound to PFV Intasome. DTG binding to the active site of the PFV intasome (PDB ID: 3S3M; orange) suggests there are three key structural features that make DTG a better able to resist mutations in and around the HIV IN active site compared to the first generation INSTIs (see text),: (1) the chelating motif of DTG (marked and outlined by a red circle) (2) the oxazinane ring on the "left-side" of DTG structure in the figure, which is conformationally flexible and occupies the same space as the target DNA (shown with a green circle), and (3) the longer linker that connects the benzyl moiety with the central pharmacophore (indicated by a blue circle). Mg2+ ions are maroon and labeled while the viral DNA is shadowed in the dark orange and line configurations (penultimate cytosine labeled dC). Catalytic DDE motif residues are depicted in gray and labeled, while residues in the active site that commonly undergo resistance mutations are depicted in cyan and labeled

Supplemental Table 1

A

	M50I	L74M	T97A	S119R	E138K	G140S	Q146L	Q146P	Q148H	Q148K	Q148R	S153Y
DTG	2.1 ± 0.9	2.2 ± 0.4	1.1 ± 0.5	2.3 ± 0.6	1.8 ± 0.4	2.7 ± 0.7	2.1 ± 0.8	0.5 ± 0.04	0.6 ± 0.1	1.0 ± 0.01	1.3 ± 0.2	2.0 ± 0.7
4c	1.3 ± 0.4	0.9 ± 0.2	1.9 ± 0.6	1.6 ± 0.6	2.1 ± 0.4	0.9 ± 0.1	3.5 ± 1.0	0.7 ± 0.04	0.5 ± 0.1	3.6 ± 0.5	2.1 ± 0.7	3.1 ± 0.4
4d	1.3 ± 0.5	1.6 ± 0.6	0.9 ± 0.02	2.1 ± 0.1	2.5 ± 0.1	1.4 ± 0.4	2.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.01	2.5 ± 0.1	2.3 ± 0.4	2.0 ± 0.4
4f	1.9 ± 0.3	2.0 ± 0.3	1.6 ± 0.6	2.6 ± 0.4	2.6 ± 0.3	1.3 ± 0.3	4.6 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	8.2 ± 2.5	2.0 ± 0.4	3.0 ± 0.1
6b	2.1 ± 0.7	4.3 ± 0.8	1.2 ± 0.1	4.2 ± 0.6	4.1 ± 0.4	2.2 ± 0.7	6.6 ± 0.4	0.8 ± 0.6	1.5 ± 0.6	6.6 ± 0.3	2.1 ± 0.1	4.6 ± 0.8
6p	1.3 ± 0.1	2.1 ± 0.1	1.4 ± 0.7	1.7 ± 0.3	1.8 ± 0.1	0.6 ± 0.3	2.7 ± 0.5	0.3 ± 0.1	0.4 ± 0.04	4.1 ± 0.3	1.4 ± 0.7	3.8 ± 0.7

B

	M50I	L74M	T97A	S119R	E138K	G140S	Q146L	Q146P	Q148H	Q148K	Q148R	S153Y
DTG - 4c	NS	0.003	NS	NS	NS	0.01	NS	< 0.001	NS	0.002	NS	0.04
DTG - 4d	NS	NS	NS	NS	0.04	0.03	NS	NS	NS	< 0.001	0.009	NS
DTG - 4f	NS	NS	NS	NS	0.02	0.02	0.008	0.02	NS	0.01	0.03	NS
DTG - 6b	NS	0.007	NS	0.004	< 0.001	NS	< 0.001	NS	NS	< 0.001	0.001	0.003
DTG - 6p	NS	NS	NS	NS	NS	0.005	NS	0.02	0.02	< 0.001	NS	0.01

Supplemental Table 1. A. The EC50 values (nM) were determined for DTG, 4c, 4d, 4f, 6b, and 6p against the INSTI-resistant mutants by using a single round infection assay, n=4. The concentrations (nM) are measured by the reductions in luciferase reporter activity in the presence of varying amounts of the inhibitors. Standard deviations follow the plus-minus sign and were calculated from the EC50 values, n=4. **B. Statistical significance in the differences in the antiviral activities of DTG and our compounds (4c, 4d, 4f, 6b, and 6p).** P values indicating statistically significant differences in the antiviral activities of DTG and our compounds (**4c, 4d, 4f, 6b,** and **6p**) for the various INSTI-resistant mutants.

Supplemental Table 2

Supplemental Table 2. Statistical significance of the antiviral data among DTG and our compounds. The Student's t test was used to calculate the statistical significance of the differences in the antiviral activities of the INSTIs. Because of multiple comparisons, P values < 0.025 were considered statistically significant when comparing the efficacies among **DTG** and **4c, 4d, 4f, 6b,** and **6p.**

Figure and Supplementary Table	*p*-Value < 0.05	*p*-Value < 0.01	*p*-Value < 0.001	Overall Comparison among INSTIs for Table			
Figure 2 Antiviral Data and Supplementary Tables 1A and 1B	DTG-4d (E138K) DTG-4f (E138K) 4d-DTG (G140S) 4f-DTG (G140S) 6p-DTG (Q146P) DTG-4f (Q146P) 6p-DTG (Q148H) DTG-4f (Q148R) DTG-4c (S153Y)	4c-DTG (L74M) DTG-4b (L74M) DTG-4b (S119R) 4c-DTG (G140S) 6p-DTG (G140S) DTG-4f (Q146L) DTG-4c (Q148K) DTG-4f (Q148K) DTG-4d (Q148R) DTG-6b (S153Y) DTG-6p (S153Y)	DTG-6b (E138K) DTG-6b (Q146L) DTG-4c (Q146P) DTG-6b (Q148K) DTG-4d (Q148K) DTG-6p (Q148K) DTG-6b (Q148R)	DTG > 4c DTG > 6b DTG > 4d DTG > 6p DTG > 4f	3 7 3 2 5	4c > DTG 6b > DTG 4d > DTG 6p > DTG 4f > DTG	2 0 1 3 1
Figure 2 Antiviral Data and Supplementary Tables 3A and 3B	6b-DTG (G140A/Q148H) 6p-DTG (Y143R/Q148H) DTG-4c (E138K/Q148K) DTG-4d (E138A/Q148R) 4c-DTG (G140C/Q148R) 4d-DTG (G140C/Q148R) 4d-DTG (G140S/Q148R) DTG-6p (G140S/Q148R) DTG-4f (Q148R/N155H)	4c-DTG (G140A/Q148H) 4d-DTG (G140A/Q148H) 6p-DTG (G140A/Q148H) 4f-DTG (G140A/Q148H) 4d-DTG (Q148H/N155H 6p-DTG (Q148H/N155H DTG-4f (E138K/Q148K) 4d-DTG (G140S/Q148K) DTG-6b (E138A/Q148R) DTG-6p (E138K/Q148R) DTG-6b (G140A/Q148R) 4d-DTG (G140A/Q148R) DTG-6p (G140A/Q148R) 4c-DTG (G140S/Q148R) DTG-6b (Q148R/N155H)	4c-DTG (Q148H/N155H) DTG-6b (E138K/Q148K) 4d-DTG (E138K/Q148K) DTG-6p (E138K/Q148K) 4c-DTG (G140A/Q148K) 4d-DTG (G140A/Q148K) 6p-DTG (G140A/Q148K) DTG-4f (G140A/Q148K) DTG-6b (G140S/Q148K) 6p-DTG (G140S/Q148K) DTG-4f (G140S/Q148K) DTG-6c (E138A/Q148R) DTG-6p (E138A/Q148R) DTG-6b (E138K/Q148R) DTG-4f (E138K/Q148R) DTG-4c (G140A/Q148R) DTG-6b (G140C/Q148R) DTG-6p (G140C/Q148R) DTG-4f (G140C/Q148R) DTG-6b (G140S/Q148R) DTG-4f (G140S/Q148R)	DTG > 4c DTG > 4d DTG > 4f DTG > 6b DTG > 6p	3 1 7 8 6	4c > DTG 4d > DTG 4f > DTG 6b > DTG 6p > DTG	5 8 1 1 5
Figure 2 Antiviral Data and Supplementary Tables 4A and 4B	DTG-4d (E92Q/N155H) DTG-4c (Y143H/N155H) DTG-4d (Y143H/N155H) DTG-4f (Y143H/N155H) DTG-6p (N155H/G163R)	DTG-4c (E92Q/N155H) DTG-4f (E92Q/N155H)	DTG-6b (E92Q/N155H) DTG-6p (E92Q/N155H) DTG-6b (N155H/G163R) DTG-4f (N155H/G163R)	DTG > 4c DTG > 4d DTG > 4f DTG > 6b DTG > 6p	2 2 3 2 2	4c > DTG 4d > DTG 4f > DTG 6b > DTG 6p > DTG	0 0 0 0 0
Figure 3 Antiviral Data and Supplementary Tables 5A and 5B	6b-DTG (E138K/G140A/Q148K) 6p-DTG (E138K/G140A/Q148K) DTG-6b (L74M/G140A/Q148R) DTG-4c (E138K/G140C/Q148R) 4c-DTG (E138A/S147G/Q148R)	4c-DTG (E138K/G140A/Q148K) 4d-DTG (E138K/G140A/Q148K) 4d-DTG (L74M/G140A/Q148R) 4d-DTG (L74M/G140C/Q148R) DTG-6b (E138K/G140C/Q148R) DTG-4d (E138K/G140C/Q148R) DTG-4f (E138K/G140C/Q148R) DTG-6b (E138A/S147G/Q148R) DTG-4f (E138A/S147G/Q148R)	DTG-4f (E138K/G140A/Q148K) 4c-DTG (L74M/G140A/Q148R) 4f-DTG (L74M/G140A/Q148R) 4c-DTG (L74M/G140C/Q148R) DTG-6b (L74M/G140C/Q148R) DTG-4f (L74M/G140C/Q148R) DTG-6p (E138K/G140C/Q148R)	DTG > 4c DTG > 4d DTG > 4f DTG > 6b DTG > 6p	1 1 4 3 2	4c > DTG 4d > DTG 4f > DTG 6b > DTG 6p > DTG	4 3 1 1 1
Figure 3 Antiviral Data and Supplementary Tables 6A and 6B	DTG-6b (T97A/G140S/Q148H) DTG-6p (E138A/G140S/Q148H) 4c-DTG (G140S/Y143R/Q148H) 4d-DTG (G140S/Y143R/Q148H) 6p-DTG (G140S/Y143R/Q148H) DTG-6b (G140S/Q148H/N155H) 6p-DTG (G140S/Q148H/G163K)	DTG-4f (T97A/G140S/Q148H) DTG-4f (E138A/G140S/Q148H) DTG-4f (G140S/Y143R/Q148H) 4d-DTG (G140S/Q148H/N155H) DTG-4f (G140S/Q148H/N155H) DTG-6b (G140S/Q148H/G163K) DTG-4f (G140S/Q148H/G163K)	4c-DTG (T97A/G140S/Q148H) 4d-DTG (T97A/G140S/Q148H) DTG-4d (T97A/G140S/Q148H) DTG-6p (E138A/G140S/Q148H) 4c-DTG (E138K/G140S/Q148H) 6b-DTG (E138K/G140S/Q148H) 4d-DTG (E138K/G140S/Q148H) 6p-DTG (E138K/G140S/Q148H) 4c-DTG (G140S/Q148H/G163K) 4d-DTG (G140S/Q148H/G163K)	DTG > 4c DTG > 4d DTG > 4f DTG > 6b DTG > 6p	0 1 5 3 2	4c > DTG 4d > DTG 4f > DTG 6b > DTG 6p > DTG	4 5 0 1 3
Figure 3 Antiviral Data and Supplementary Tables 7A and 7B	DTG-4d (T66I/T97A/E157Q) DTG-4f (T66I/T97A/E157Q) XZ434-6p (T97A/Y143R/N155H) DTG-4c (T97A/Q148H/N155H) 4c-DTG (G140S/Y143R/N155H) DTG-4d (E92Q/N155H/G163R) DTG-6p (E92Q/N155H/G163R)	DTG-6p (T66I/T97A/E157Q) 4c-DTG (T97A/Y143R/N155H) 4d-DTG (T97A/Y143R/N155H) DTG-4f (T97A/Q148H/N155H) 6b-DTG (G140S/Y143R/N155H) DTG-6b (E92Q/N155H/G163R) DTG-4f (E92Q/N155H/G163R)	4c-DTG (T97A/Y143R/Q148H) 6b-DTG (T97A/Y143R/Q148H) 4d-DTG (T97A/Y143R/Q148H) 6p-DTG (T97A/Y143R/Q148H) 6p-DTG (G140S/Y143R/N155H)	DTG > 4c DTG > 4d DTG > 4f DTG > 6b DTG > 6p	1 2 3 1 2	4c > DTG 4d > DTG 4f > DTG 6b > DTG 6p > DTG	3 2 0 2 3

Supplemental Table 3

A

	T66I/ E157Q	G140A/ Q148H	Y143R/ Q148H	Q148H/ N155H	E138K/ Q148K	G140A/ Q148K	G140S/ Q148K	E138A/ Q148R	E138K/ Q148R	G140A/ Q148R	G140C/ Q148R	G140S/ Q148R	Q148R/ N155H
DTG	0.5 ± 0.1	3.9 ± 0.7	0.8 ± 0.2	4.0 ± 0.6	25.0 ± 2.1	450.7 ± 58.8	2.3 ± 0.2	4.4 ± 0.8	3.9 ± 0.1	4.1 ± 0.3	2.9 ± 0.6	26.2 ± 6.8	7.1 ± 2.0
4c	0.4 ± 0.1	1.6 ± 0.4	0.6 ± 0.1	0.9 ± 0.3	29.5 ± 2.7	43.1 ± 1.8	2.8 ± 0.9	9.4 ± 1.1	4.2 ± 1.9	2.3 ± 0.4	1.5 ± 0.4	4.8 ± 0.6	9.6 ± 2.1
4d	0.4 ± 0.1	0.9 ± 0.1	0.6 ± 0.04	1.1 ± 0.2	16.0 ± 1.2	37.7 ± 1.2	1.6 ± 0.1	5.9 ± 0.7	3.0 ± 0.7	2.6 ± 0.5	1.5 ± 0.6	12.9 ± 1.2	7.5 ± 0.1
4f	0.5 ± 0.1	1.5 ± 0.1	0.8 ± 0.1	4.3 ± 1.7	127.0 ± 16.8	789.0 ± 26.7	5.1 ± 0.4	5.1 ± 0.8	10.8 ± 0.8	4.3 ± 1.1	61.5 ± 1.1	181.6 ± 12.0	10.6 ± 1.7
6b	0.5 ± 0.1	2.7 ± 0.1	0.5 ± 0.02	3.1 ± 0.6	134.4 ± 1.8	392.9 ± 13.9	3.8 ± 0.3	26.7 ± 4.9	16.7 ± 1.6	14.1 ± 2.6	9.0 ± 1.5	64.9 ± 7.3	31.3 ± 8.0
6p	0.3 ± 0.2	1.2 ± 0.3	0.4 ± 0.2	2.1 ± 0.1	45.3 ± 2.5	54.1 ± 6.7	0.84 ± 0.2	9.8 ± 0.3	14.6 ± 2.0	6.3 ± 1.1	6.8 ± 0.9	46.5 ± 11.9	5.6 ± 0.4

B

	T66I/ E157Q	G140A/ Q148H	Y143R/ Q148H	Q148H/ N155H	E138K/ Q148K	G140A/ Q148K	G140S/ Q148K	E138A/ Q148R	E138K/ Q148R	G140A/ Q148R	G140C/ Q148R	G140S/ Q148R	Q148R/ N155H
DTG - 4c	NS	0.003	NS	< 0.001	0.04	< 0.001	NS	< 0.001	NS	< 0.001	0.01	0.008	NS
DTG - 4d	NS	0.003	NS	0.001	< 0.001	< 0.001	0.002	0.03	NS	0.004	0.02	0.03	NS
DTG - 4f	NS	0.006	NS	NS	0.001	< 0.001	< 0.001	NS	< 0.001	NS	< 0.001	< 0.001	0.04
DTG - 6b	NS	0.04	NS	NS	< 0.001	NS	< 0.001	0.002	< 0.001	0.004	< 0.001	< 0.001	0.007
DTG - 6p	NS	0.002	0.03	0.007	< 0.001	< 0.001	< 0.001	< 0.001	0.002	0.002	< 0.001	0.03	NS

Supplemental Table 3 A. The EC50 values (nM) were determined for DTG, 4c, 4d, 4f, 6b, and 6p against the INSTI-resistant mutants by using a single round infection assay, n=4. The concentrations (nM) are measured by the reductions in luciferase reporter activity in the presence of varying amounts of the inhibitors. Standard deviations follow the plus-minus sign and were calculated from the EC50 values, n=4. **B. Statistical significance in the differences in the antiviral activities of DTG and our compounds (4c, 4d, 4f, 6b, and 6p).** P values indicating statistically significant differences in the antiviral activities of DTG and our compounds (**4c, 4d, 4f, 6b,** and **6p**) for the various INSTI-resistant mutants.

Supplemental Table 4

A

	E92Q/ N155H	G140S/ N155H	Y143H/ N155H	Y143R/ N155H	N155H/ G163R
DTG	1.8 ± 0.2	2.0 ± 1.0	1.7 ± 0.7	2.5 ± 0.8	1.6 ± 0.4
4c	4.7 ± 0.8	1.8 ± 0.3	2.8 ± 0.5	1.9 ± 0.6	2.6 ± 0.7
4d	2.8 ± 0.6	2.3 ± 0.4	3.6 ± 0.1	1.7 ± 0.2	2.1 ± 0.6
4f	22.3 ± 5.2	2.3 ± 0.6	3.7 ± 0.9	3.8 ± 0.9	5.2 ± 0.3
6b	5.4 ± 0.4	2.8 ± 0.6	1.5 ± 0.4	2.8 ± 0.3	7.0 ± 0.6
6p	4.5 ± 0.4	0.9 ± 0.3	1.5 ± 0.6	1.8 ± 0.2	2.3 ± 0.1

B

	E92Q/ N155H	G140S/ N155H	Y143H/ N155H	Y143R/ N155H	N155H/ G163R
DTG - 4c	0.004	NS	0.05	NS	NS
DTG - 4d	0.04	NS	0.01	NS	NS
DTG - 4f	0.004	NS	0.01	NS	< 0.001
DTG - 6b	< 0.001	NS	NS	NS	< 0.001
DTG - 6p	< 0.001	NS	NS	NS	0.04

Supplemental Table 4. A. The EC50 values (nM) were determined for DTG, 4c, 4d, 4f, 6b, and 6p against the INSTI-resistant mutants by using a single round infection assay, n=4. The concentrations (nM) are measured by the reductions in luciferase reporter activity in the presence of varying amounts of the inhibitors. Standard deviations follow the plus-minus sign and were calculated from the EC50 values, n=4. **B. Statistical significance in the differences in the antiviral activities of DTG and our compounds (4c, 4d, 4f, 6b, and 6p).** P values indicating statistically significant differences in the antiviral activities of DTG and our compounds (**4c, 4d, 4f, 6b,** and **6p**) for the various INSTI-resistant mutants.

Supplemental Table 5

A

	E138K/G140A/ Q148K	L74M/G140A/ Q148R	L74M/G140C/ Q148R	E138K/G140C/ Q148R	E138A/S147G/ Q148R
DTG	212.1 ± 46.0	12.0 ± 0.2	10.2 ± 1.3	5.3 ± 1.0	5.5 ± 1.3
4c	18.3 ± 6.3	6.3 ± 0.9	3.3 ± 0.8	11.2 ± 2.5	2.7 ± 0.5
4d	11.6 ± 3.0	6.0 ± 1.6	5.5 ± 1.3	11.3 ± 2.1	3.7 ± 0.9
4f	500.1 ± 64.0	3.8 ± 0.3	42.1 ± 4.9	111.1 ± 22.5	18.2 ± 4.2
6b	138.1 ± 23.5	25.3 ± 6.7	53.8 ± 4.9	35.8 ± 5.4	12.9 ± 2.5
6p	89.6 ± 6.6	13.6 ± 1.8	12.8 ± 2.4	22.5 ± 1.3	3.9 ± 0.5

B

	E138K/G140A/ Q148K	L74M/G140A/ Q148R	L74M/G140C/ Q148R	E138K/G140C/ Q148R	E138A/S147G/ Q148R
DTG - 4c	0.003	< 0.001	< 0.001	0.01	0.02
DTG - 4d	0.003	0.005	0.002	0.006	NS
DTG - 4f	< 0.001	< 0.001	< 0.001	0.003	0.006
DTG - 6b	0.04	0.03	< 0.001	0.001	0.004
DTG - 6p	0.01	NS	NS	< 0.001	NS

Supplemental Table 5 A. The EC50 values (nM) were determined for DTG, 4c, 4d, 4f, 6b, and 6p against the INSTI-resistant mutants by using a single round infection assay, n=4. The concentrations (nM) are measured by the reductions in luciferase reporter activity in the presence of varying amounts of the inhibitors. Standard deviations follow the plus-minus sign and were calculated from the EC50 values, n=4. **B. Statistical significance in the differences in the antiviral activities of DTG and our compounds (4c, 4d, 4f, 6b, and 6p).** P values indicating statistically significant differences in the antiviral activities of DTG and our compounds (**4c, 4d, 4f, 6b,** and **6p**) for the various INSTI-resistant mutants.

Supplemental Table 6

A

	T97A/G140S/ Q148H	E138A/G140S/ Q148H	E138K/G140S/ Q148H	G140S/Y143R/ Q148H	G140S/Q148H/ N155H	G140S/Q148H/ G163K
DTG	55.9 ± 3.0	13.8 ± 4.8	68.2 ± 2.0	7.7 ± 2.0	77.9 ± 15.9	24.3 ± 1.1
4c	27.7 ± 3.7	11.3 ± 1.3	11.1 ± 0.9	3.6 ± 1.2	93.4 ± 9.2	9.8 ± 2.3
4d	20.4 ± 1.0	7.3 ± 0.4	7.7 ± 1.3	4.6 ± 0.5	21.6 ± 2.2	11.4 ± 1.0
4f	124.9 ± 21.4	97.4 ± 20.0	55.9 ± 9.9	36.1 ± 9.3	142.3 ± 24.3	82.4 ± 14.5
6b	124.9 ± 23.9	47.7 ± 1.9	31.2 ± 1.9	9.2 ± 2.0	116.9 ± 19.7	31.4 ± 1.9
6p	99.0 ± 3.7	33.7 ± 8.6	28.8 ± 1.2	4.1 ± 0.7	71.6 ± 16.0	12.2 ± 0.2

B

	T97A/G140S/ Q148H	E138A/G140S/ Q148H	E138K/G140S/ Q148H	G140S/Y143R/ Q148H	G140S/Q148H/ N155H	G140S/Q148H/ G163K
DTG - 4c	< 0.001	NS	< 0.001	0.02	NS	< 0.001
DTG - 4d	< 0.001	NS	< 0.001	0.05	0.005	< 0.001
DTG - 4f	0.007	0.003	NS	0.007	0.006	0.004
DTG - 6b	0.01	< 0.001	< 0.001	NS	0.02	0.002
DTG - 6p	< 0.001	0.01	< 0.001	0.03	NS	0.03

Supplemental Table 6. A. The EC50 values (nM) were determined for DTG, 4c, 4d, 4f, 6b, and 6p against the INSTI-resistant mutants by using a single round infection assay, n=4. The concentrations (nM) are measured by the reductions in luciferase reporter activity in the presence of varying amounts of the inhibitors. Standard deviations follow the plus-minus sign and were calculated from the EC50 values, n=4. **B. Statistical significance in the differences in the antiviral activities of DTG and our compounds (4c, 4d, 4f, 6b, and 6p).** P values indicating statistically significant differences in the antiviral activities of DTG and our compounds (**4c, 4d, 4f, 6b,** and **6p**) for the various INSTI-resistant mutants.

Supplemental Table 7

A

	T66I/T97A/ E157Q	T97A/Y143R/ Q148H	T97A/Y143R/ N155H	T97A/Q148H/ N155H	G140S/Y143R/ N155H	E92Q/N155H/ G163R
DTG	0.5 ± 0.1	1.5 ± 0.1	8.5 ± 1.5	2.4 ± 0.7	2.6 ± 0.3	3.8 ± 0.7
4c	0.5 ± 0.2	0.5 ± 0.2	3.8 ± 1.1	1.1 ± 0.2	1.3 ± 0.6	4.9 ± 0.6
4d	1.3 ± 0.5	0.4 ± 0.1	2.7 ± 0.7	2.4 ± 0.4	2.4 ± 0.6	5.4 ± 0.2
4f	3.2 ± 1.0	2.0 ± 0.5	10.1 ± 2.9	4.4 ± 0.6	1.9 ± 0.5	29.8 ± 5.3
6b	0.9 ± 0.3	0.7 ± 0.1	8.3 ± 1.7	2.6 ± 0.4	1.6 ± 0.1	21.8 ± 4.0
6p	0.8 ± 0.04	0.5 ± 0.03	4.6 ± 2.0	2.2 ± 0.1	1.0 ± 0.3	10.0 ± 2.5

B

	T66I/T97A/ E157Q	T97A/Y143R/ Q148H	T97A/Y143R/ N155H	T97A/Q148H/ N155H	G140S/Y143R/ N155H	E92Q/N155H/ G163R
DTG - 4c	NS	< 0.001	0.003	0.03	0.02	NS
DTG - 4d	0.05	< 0.001	0.002	NS	NS	0.02
DTG - 4f	0.01	NS	NS	0.005	NS	0.002
DTG - 6b	NS	< 0.001	NS	NS	0.004	0.002
DTG - 6p	0.005	< 0.001	0.02	NS	< 0.001	0.01

Supplemental Table 7 A. The EC50 values (nM) were determined for DTG, 4c, 4d, 4f, 6b, and 6p against the INSTI-resistant mutants by using a single round infection assay, n=4. The concentrations (nM) are measured by the reductions in luciferase reporter activity in the presence of varying amounts of the inhibitors. Standard deviations follow the plus-minus sign and were calculated from the EC50 values, n=4. **B. Statistical significance in the differences in the antiviral activities of DTG and our compounds (4c, 4d, 4f, 6b, and 6p).** P values indicating statistically significant differences in the antiviral activities of DTG and our compounds (**4c, 4d, 4f, 6b,** and **6p**) for the various INSTI-resistant mutants.

Supplemental Table 8

Mutant	% of WT Activity (±SD)
M50I	90.4 ± 20.5
T66I	79.4 ± 6.7
L74M	89.6 ± 18.3
T97A	92.8 ± 4.0
G118R	23.0 ± 2.2
S119R	78.6 ± 15.5
E138K	80.9 ± 2.7
G140S	72.8 ± 12.1
Q146L	64.6 ± 11.4
Q146P	74.7 ± 9.3
Q148H	51.7 ± 5.4
Q148K	14.2 ± 0.8
Q148R	43.3 ± 6.5
S153Y	64.7 ± 12.0
H51Y/R263K	13.8 ± 3.9
T66I/E157Q	85.9 ± 7.6
E92Q/N155H	71.4 ± 6.7
E138A/Q148R	75.2 ± 16.1
E138K/Q148K	49.4 ± 7.5
E138K/Q148R	59.9 ± 5.4
E138K/R263K	55.2 ± 8.0
G140A/Q148H	8.8 ± 1.1
G140A/Q148K	12.3 ± 1.3
G140A/Q148R	48.6 ± 8.1
G140C/Q148R	28.8 ± 2.0
G140S/Q148H	66.0 ± 16.8
G140S/Q148K	44.6 ± 8.5
G140S/Q148R	32.6 ± 7.0
G140S/N155H	19.5 ± 3.8
Y143H/N155H	13.7 ± 3.3
Y143R/Q148H	2.6 ± 0.2
Y143R/N155H	33.9 ± 6.5
Q148H/N155H	7.3 ± 2.9
Q148R/N155H	13.8 ± 4.1
N155H/G163R	55.1 ± 6.9
T66I/T97A/E157Q	72 ± 4.5
L74M/G140A/Q148R	32.5 ± 4.4
L74M/G140C/Q148R	29.4 ± 7.3
E92Q/N155H/G163R	55.2 ± 11.6
T97A/G140S/Q148H	55.9 ± 13.1
T97A/Y143R/Q148H	5.2 ± 1.3
T97A/Y143R/N155H	15.4 ± 2.1
T97A/Q148H/N155H	6.6 ± 1.7
E138A/G140S/Q148H	85.2 ± 10.2
E138A/S147G/Q148R	83.7 ± 13.1
E138K/G140A/Q148K	42.9 ± 7.5
E138K/G140C/Q148R	44.4 ± 7.1
E138K/G140S/Q148H	54.4 ± 15.9
G140S/Y143R/Q148H	52.3 ± 1.1
G140S/Y143R/N155H	2.9 ± 0.8
G140S/Q148H/N155H	63 ± 14.9
G140S/Q148H/G163K	49.6 ± 17.2
Mock	0 ± 0

Supplemental Table 8. Replication capacity of the INSTI-resistant mutants using a single-round infection assay. The numerical values of the replication capacities of the INSTI-resistant mutants used in this study were measured using INSTI-resistant mutant vectors in a single round infection assay. The luciferase activity of the WT virions was set to 100, and the infectivity of the mutant vectors (adjusted for the amount of p24/Gag used in the assay) was measured relative to WT infectivity. Error bars represent the standard deviations of independent experiments, n=4.

Supplemental Table 9

Overall Comparison

DTG > 4c	10	4c > DTG	18
DTG > 4d	10	4d > DTG	19
DTG > 4f	27	4f > DTG	3
DTG > 6b	24	6b > DTG	5
DTG > 6p	16	6p > DTG	15

***p*-Value < 0.05**

DTG > 4c	5	4c > DTG	4
DTG > 4d	6	4d > DTG	4
DTG > 4f	6	4f > DTG	1
DTG > 6b	3	6b > DTG	2
DTG > 6p	4	6p > DTG	7

***p*-Value < 0.01**

DTG > 4c	2	4c > DTG	6
DTG > 4d	2	4d > DTG	9
DTG > 4f	13	4f > DTG	1
DTG > 6b	10	6b > DTG	1
DTG > 6p	4	6p > DTG	3

***p*-Value < 0.001**

DTG > 4c	3	4c > DTG	8
DTG > 4d	2	4d > DTG	6
DTG > 4f	8	4f > DTG	1
DTG > 6b	12	6b > DTG	2
DTG > 6p	7	6p > DTG	5

Supplemental Table 9. Overall Comparison of the statistical significance of the antiviral data among DTG and 4c, 4d, 4f, 6b, and 6p. The Student's t test was used to calculate the statistical significance of the differences in the antiviral activities of DTG and our compounds. The P values < 0.025, < 0.01, and < 0.001 between **DTG** and **4c, 4d, 4f, 6b, and 6p** were used to decide which INSTIs were more broadly efficacious against the mutants.