Supporting Information

MassChemSite for in-depth forced degradation analysis of PARP inhibitors olaparib, rucaparib, and niraparib.

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MANUAL MS/MS SPECTRA CHARACTERIZATION OF RUCAPARIB DPs

DP-R3 and DP-R3'

The fragmentation pattern characterization of **DP-R3** and **DP-R3'** included the product ions at *m/z* 325.0978 (loss of CH_3NH_2 from $[M + H]^+$), at *m/z* 308.0717 (loss of NH_3 from *m/z* 325.0978), at *m/z* 296.0706 (loss of CH_2NH from *m/z* 325.0978), at *m/z* 280.0742 (loss of CH_3NO from *m/z* 325.0978), at *m/z* 268.0755 (loss of CO from *m/z* 296.0706), at *m/z* 254.0608 (loss of CH_2 from *m/z* 268.0775), at *m/z* 236.0505 (loss of H_2O from *m/z* 254.0608), at *m/z* 226.0659 (loss of CO from *m/z* 254.0608), at 208.0554 (loss of H_2O from *m/z* 226.0659), at *m/z* 118.0414 ($C_8H_6O^{\bullet+}$) and at *m/z* 90.0465 ($C_7H_6^{\bullet+}$).

DP-R4

The fragmentation pattern characterization of **DP-R4** included the product ions at *m/z* 341.0925 (loss of CH_3NH_2 from $[M + H]^+$), at 308.0954 (radical loss of OOH from *m/z* 341.0925), at *m/z* 279.0691 (loss of CH_2NH from *m/z* 308.0954), at *m/z* 253.0530 (loss of C_2H_2 from *m/z* 279.0691), at *m/z* 225.0582 (loss of CO from *m/z* 253.0530), at *m/z* 118.0414 ($C_8H_6O^{\bullet+}$) and at *m/z* 90.0467 ($C_7H_6^{\bullet+}$).

DP-R2 and **DP-R2'**

The fragmentation pattern characterization of **DP-R2** and **DP-R2'** included the product ions at *m/z* 325.0982 (neutral loss of CH_3NH_2 from $[M + H]^+$), at *m/z* 308.0952 (radical loss of OH from *m/z* 325.0982), at *m/z* 292.1005 (radical loss of OOH from *m/z* 325.0982), at 279.0690 (radical loss of OH plus loss of CH_2NH from *m/z* 325.0982), at *m/z* 263.0739 (loss of CH_3NO from *m/z* 308.0952), at *m/z* 249.0943 (loss of (O_2-2H) from *m/z* 279.0690), at *m/z* 235.0792 (loss of CO from *m/z* 263.0739) and at *m/z* 90.0465 ($C_7H_6^{\bullet+}$).

DP-R1

The fragmentation pattern characterization of **DP-R1** included the product ions at *m/z* 309.1035 (due to loss of CH_3NH_2 from $[M + H]^+$) and at *m/z* 280.0774 (loss of CH_2NH from *m/z* 309.1035), at *m/z* 252.0791 (loss CO from *m/z* 280.0774), at *m/z* 224.0872 (loss CO from *m/z* 252.0791), at *m/z* 203.0596 ($C_{11}H_8FN_2O^+$), at *m/z* 165.0585 ($C_9H_8FNO^{\bullet+}$) and at *m/z* 91.0541 ($C_7H_7^+$).

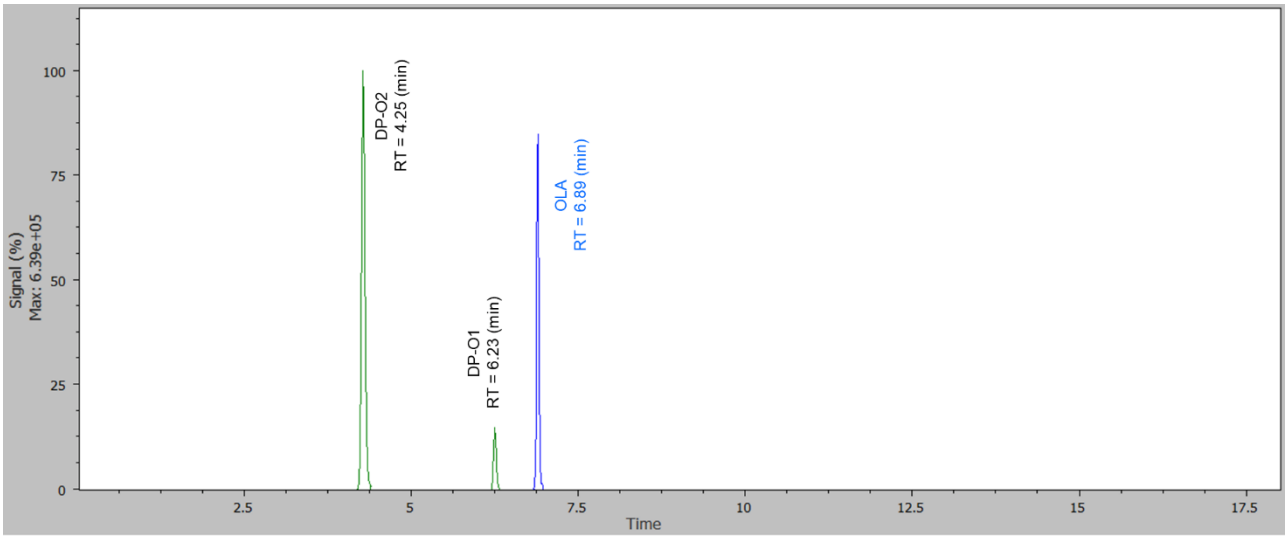


Figure S1. Chromatogram of basic forced degradation (1 M NaOH, 60°C) of olaparib acquired after 270 min of the reaction. **DP-O1** detected at 6.23 min, **DP-O2** at 4.25 min, and olaparib (OLA**)** at 6.89 min. Image from MassChemSite.

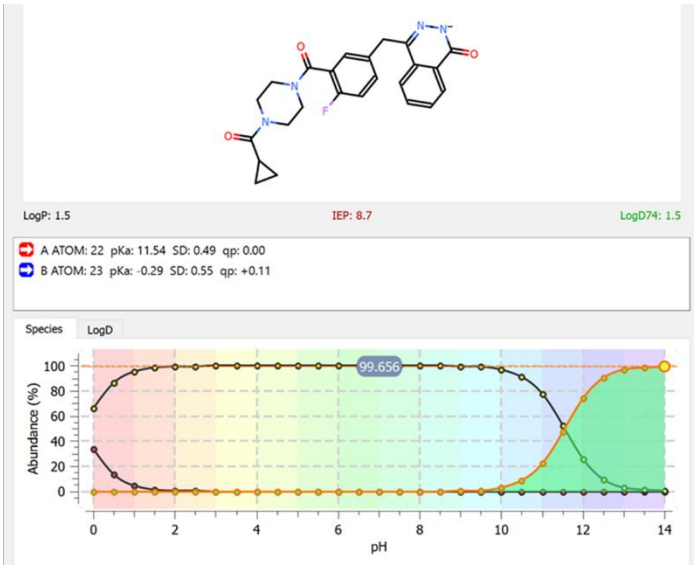


Figure S2. Prediction of the pKa value of olaparib provided by MoKa [37].

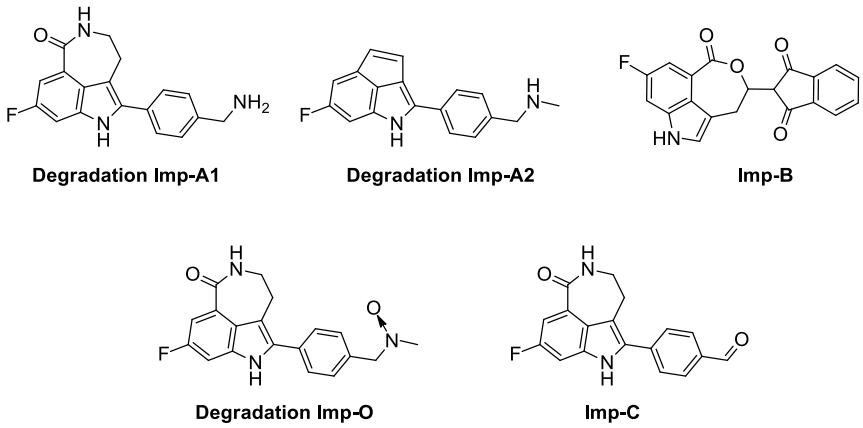


Figure S3. Structure of the degradation products (DPs) of rucaparib reported in Palakeeti et al.[24]

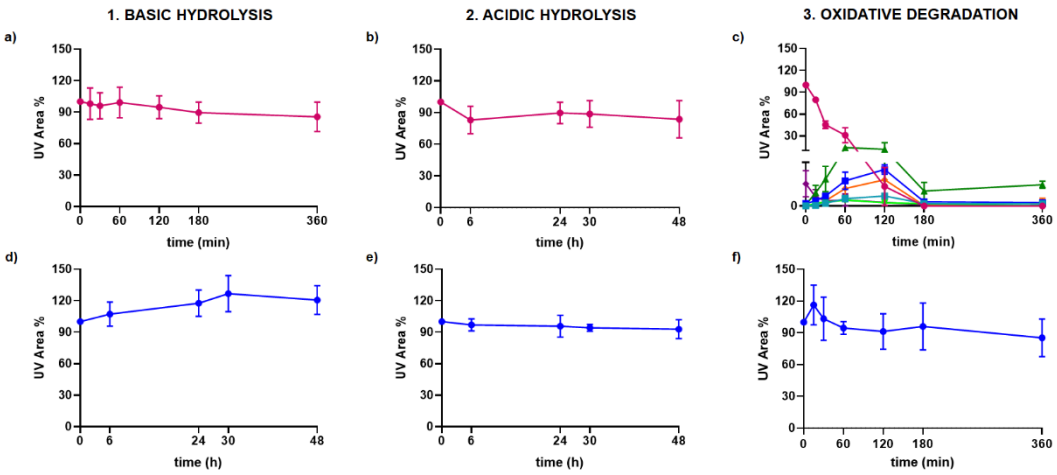


Figure S4. Trend of rucaparib, rucaparib DPs and niraparib under the studied stress conditions. Plot of UV area % *vs* time for (1) basic hydrolysis (1M NaOH, 60 °C) of rucaparib (a) and niraparib (d); (2) acidic hydrolysis (1M HCl, 60 °C), of rucaparib (b) and niraparib (e); and (3) oxidative degradation (15% H_2O_2, 60 °C) of rucaparib (c) and niraparib (f). Rucaparib (purple), **DP-R1** (violet), **DP-R2** (light green), **DP-R2'** (dark green), **DP-R3** (cyan), **DP-R3'** (blue), **DP-R4** (orange) and niraparib (blue). All the compounds are detected as $[M + H]^+$; UV signal is extracted at 254 nm. Data analysis was performed with MCS (version 3.1). Plots prepared with GraphPad Prism (version 8.4.3).

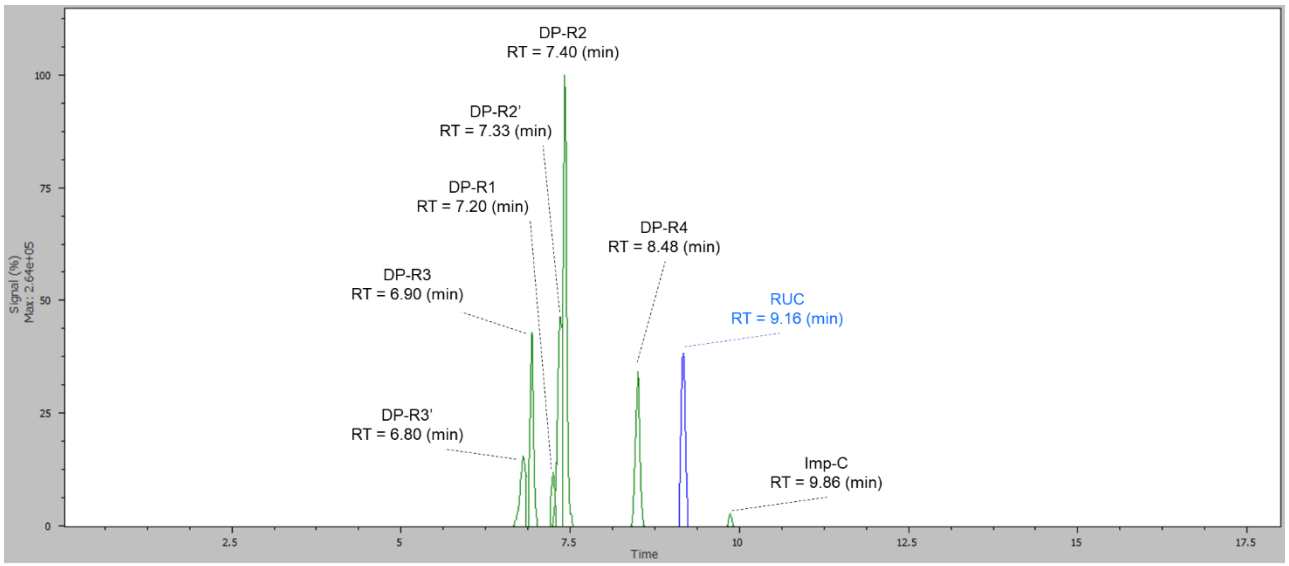


Figure S5. Chromatogram of oxidative forced degradation of rucaparib acquired after 60 min of the reaction (15% H_2O_2, 60 °C). **DP-R1** detected at 7.20 min, **DP-R2** at 7.40 min, **DP-R2'** at 7.33 min, **DP-R3** at 6.90 min, **DP-R3'** at 6.80 min, **DP-R4** at 8.48 min, **Imp-C** at 9.86 min and, rucaparib (RUC) at 9.16 min. Image from MassChemSite.

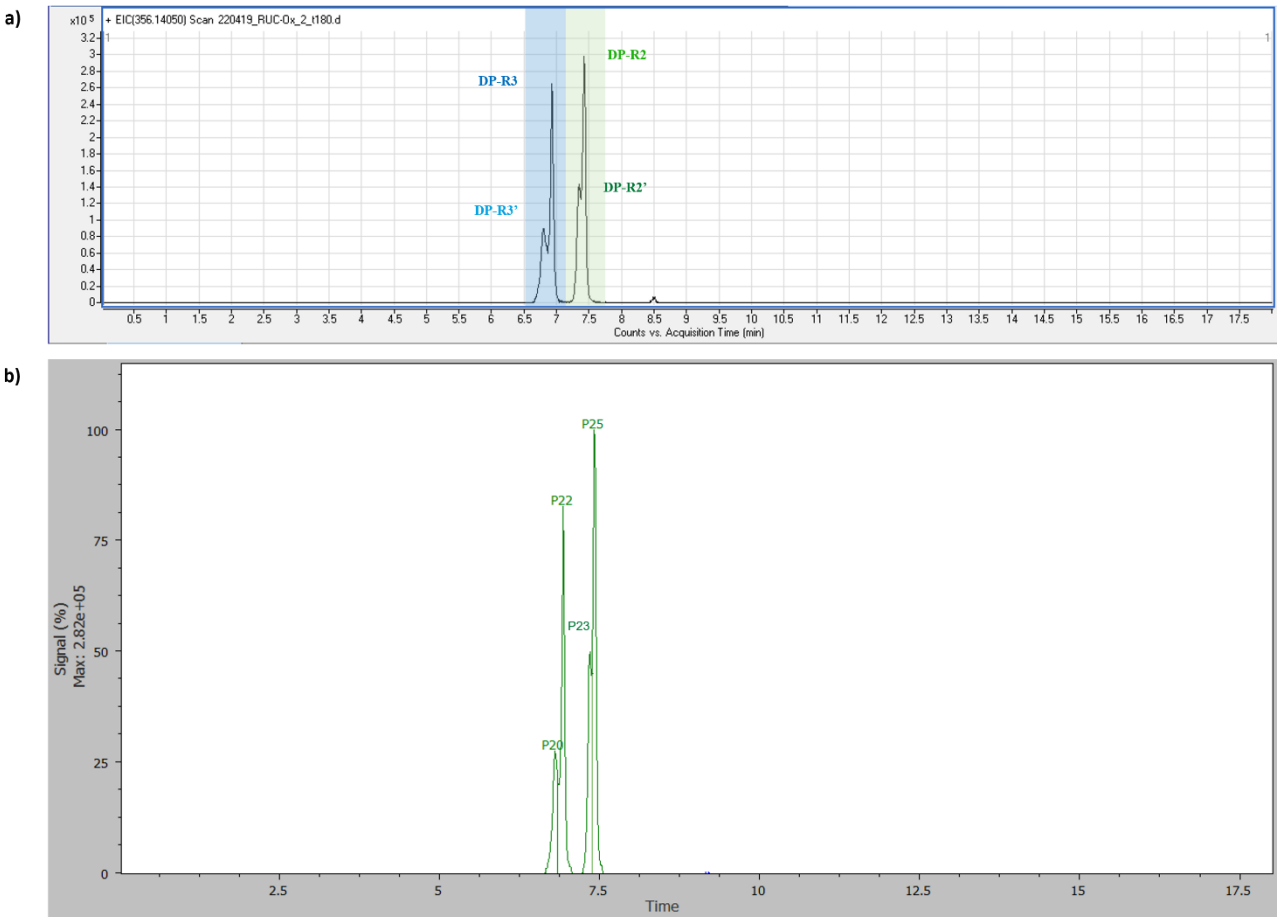


Figure S6. a) Extracted ion chromatogram (EIC) at *m/z* (356.1405 ± 5 ppm); chromatogram peaks of **DP-R2** (*RT* = 7.40 min) and **DP-R2'** (*RT* = 7.33 min) are highlighted in green while peaks related to **DP-R3** (*RT* = 6.90 min) and **DP-R3'** (*RT* = 6.80 min) are showed in blue. b) Peak detection of **DP-R2** (P25), **DP-R2'** (P23), **DP-R3** (P22) and **DP-R3'** (P20) performed by MCS software.

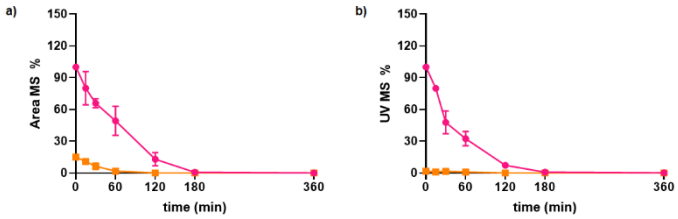


Figure S7. Trend of MS area % (a) and UV area % *vs* time (b) of rucaparib (purple) and **Imp-C** (orange) observed under the oxidative stress conditions (15% H_2O_2, 60 °C). All the compounds are detected as $[M + H]^+$; UV signal is extracted at 254 nm. Data analysis was performed with MCS (version 3.1). Plots prepared with GraphPad Prism (version 8.4.3).

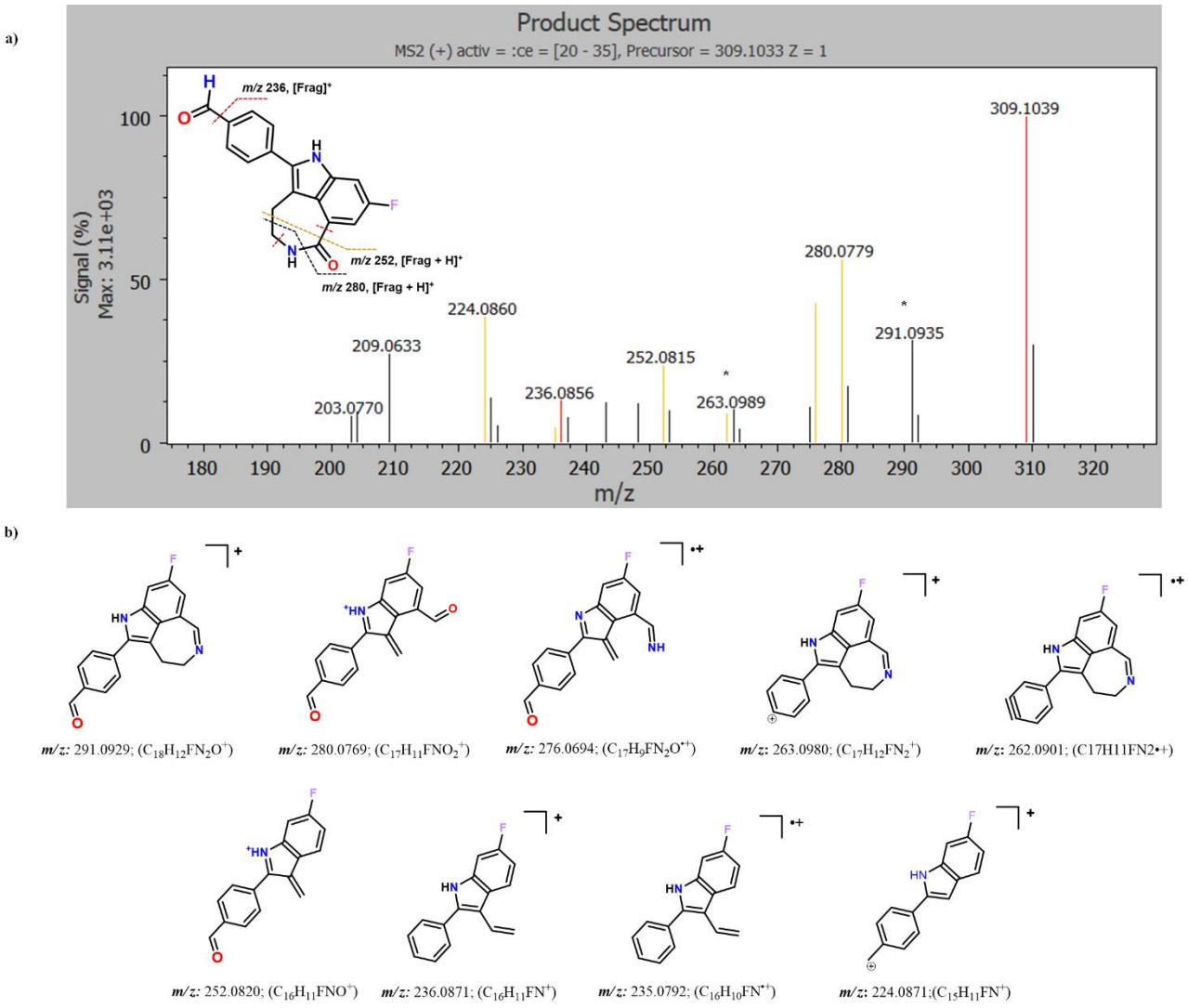


Figure S8. a) MS/MS spectrum of Imp-C (*m/z* 309.1039, *RT* = 9.86 min) analyzed by MCS. Representation of the main fragmentations identified by the software are reported (top-left). *Fragment ions identified by manual investigation. b) Structures of fragment ions of Imp-C identified.

Table S1. Degradation conditions used for olaparib degradation studies and detected DPs previously reported in literature.

Reference	Basic	Acidic	Neutral	Oxidative	Dry Heat	Photolytic
Thummar et al. [20]	1 mg/mL of drug stock solution - 0.2 M NaOH at 70°C for 10 h. (**DP-O1** and **DP-O2**)	1 mg/mL of drug stock solution - 1M HCl at 70°C for 4 h. (**DP-O1** and **DP-O2**)	1 mg/mL of drug stock solution - water at 70°C for 7 days. (NDP)	1 mg/mL of drug stock solution - 15% w/v H_2O_2 for 120 h, rt. (**DP-O3** and **DP-O4**)	solid drug (1 mm thickness layer) at 85 ◦C for 7 days. (NDP)	drug in solid state and solution state (1 mg/mL) - Up to 200 W h/m^2 UV light and up to 1.2 million lux hr (vis). (NDP)
Kallepalli et al. [21]	1 mg/mL of drug stock solution - 1M NaOH at 80°C for 1 h (NDP)	1 mg/mL of drug stock solution -1M HCl at 80°C for 1h (**DP-O1** and **DP-O2**)	(NS)	1 mg/mL of drug stock solution - 30% H_2O_2 at 80°C for 1 h (**DP-OX** *m/z* 367)	(NS)	(NS)
Khedr et al. [22]	0.666 mg/mL of drug solution - 0.33 M NaOH at 60°C for 30 min, capped. (**DP-O1** and **DP-O2**)	0.666 mg/mL of drug solution - 0.33 M HCl at 60°C for 30 min, capped. (NDP)	0.500 mg/mL of drug solution ACN:H_2O (1:1, v:v %) at 95°C for 30 min, capped. (NDP)	0.500 mg/mL of drug solution - 15% H_2O_2 w/w at 60°C for 30 min, capped. (NDP)	(NS)	0.1 mm thickness layer of powder drug subjected to UV irradiation from a distance of 5 cm for 24 h. (**DP-O1** and **DP-O2**)
Kavitapu et al. [23]	20 mg/mL of drug solution - 1 M NaOH at 60°C for 20 h, capped. (**DP-O1**, **DP-O2** and **DP-O5**)	20 mg/mL of drug solution - 1 M HCl at 60°C for 20 h, capped (**DP-O1**, **DP-O2** and **DP-O5**)	(NS)	20 mg/mL of drug solution - 30% H_2O_2 at rt for 25 h, capped under dark. (NDP)	drug in both solid and solution state form at 80°C for 48 h. (NDP)	Up to 200 W h/m^2 UV light and up to 1.2 million lux hr (vis). On solid and solution of ola. (NDP)

*(NDP): no degradants observed; (NS): no-studied condition; rt: room temperature; **DP-Os**: olaparib degradation products.

Table S2. IUPAC names and mass error (ppm) of identified DPs and impurities of olaparib and rucaparib suggested by MCS and by manual inspection.

	IUPAC name	**ppm mass error**
DP-O1	2-fluoro-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl) benzoic acid	2.34 ppm
DP-O2	4-(4-fluoro-3-(piperazin-1-carbonyl)benzyl)phthalazin-1(2*H*)-one	2.99 ppm
DP-O4	4-(3-(4-(cyclopropancarbonyl)-1,2,3,4-tetrahydropyrazine-1-carbonyl)-4-fluorobenzyl)phthalazine-1(2*H*)-one	2.54 ppm
DP-R1	Proposed by MCS: 8-fluoro-3-hydroxy-2-(4-((methylamino)methyl)phenyl)-4,5-dihydro-1*H*-azepino[5,4,3-*cd*]indol-6(3*H*)-one Proposed by manual inspection: 7-hydroxy- or 9-hydroxy- 8-fluoro-2-(4-((methylamino)methyl)phenyl)-4,5-dihydro-1*H*-azepino[5,4,3-*cd*]indol-6(3*H*)-one	-1.18 ppm
DP-R2 and DP-R2'	Proposed by MCS: 8-fluoro-2-(4-(dihydroxy(methylamino)methyl)phenyl)-4,5-dihydro-1*H*-azepino[5,4,3-*cd*]indol-6(3*H*)-one Proposed by manual inspection: 8-fluoro-9-hydroxy-2-(4-(hydroxy(methylamino)methyl)phenyl)-4,5-dihydro-1*H*-azepino[5,4,3-*cd*]indol-6(3*H*)-one and 8-fluoro-7-hydroxy-2-(4-(hydroxy(methylamino)methyl)phenyl)-4,5-dihydro-1*H*-azepino[5,4,3-*cd*]indol-6(3*H*)-one	-0.28 ppm
DP-R3 and DP-R3'	Proposed by MCS: 7-hydroxy-8-fluoro-2-(4-(hydroxy(methylamino)methyl)phenyl)-4,5-dihydro-1*H*-azepino[5,4,3-*cd*]indol-6(3*H*)-one and 9-hydroxy-8-fluoro-2-(4-(hydroxy(methylamino)methyl)phenyl)-4,5-dihydro-1*H*-azepino[5,4,3-*cd*]indol-6(3*H*)-one Proposed by manual inspection: 8-fluoro-3,9-dihydroxy-2-(4-((methylamino)methyl)phenyl)-4,5-dihydro-1*H*-azepino[5,4,3-*cd*]indol-6(3*H*)-one and 8-fluoro-3,7-dihydroxy-2-(4-((methylamino)methyl)phenyl)-4,5-dihydro-1*H*-azepino[5,4,3-*cd*]indol-6(3*H*)-one	0 ppm
DP-R4	Proposed by MCS: 8-fluoro-7,9-dihydroxy-2-(4-(hydroxy(methylamino)methyl)phenyl)-4,5-dihydro-1*H*-azepino[5,4,3-*cd*]indol-6(3*H*)-one Proposed by manual inspection: 8-fluoro-3,7,9-trihydroxy-2-(4-((methylamino)methyl)phenyl)-4,5-dihydro-1*H*-azepino[5,4,3-*cd*]indol-6(3*H*)-one	0 ppm
Rucaparib Imp-C	4-(8-fluoro-6-oxo-3,4,5,6-tetrahydro-1*H*-azepino[5,4,3-*cd*]indol-2-yl)benzaldehyde	1.62 ppm

Table S3. Literature data of static dielectric constant of ACN:H_2O mixtures at 60 °C.[40]

Acetonitrile % v:v	ε_r
25.0	59.78
12.5	63.81
0	66.82

40. Gagliardi, L. G.; Castells, C. B.; Ràfols, C.; Rosés, M.; Bosch, E., Static Dielectric Constants of Acetonitrile/Water Mixtures at Different Temperatures and Debye−Hückel A and a0B Parameters for Activity Coefficients. *Journal of Chemical & Engineering Data* **2007,** *52* (3), 1103-1107.

Table S4. Degradation conditions used for rucaparib degradation studies and detected DPs previously reported in literature.

Article	Basic	Acidic	Neutral	Oxidative	Dry Heat	Photolytic	Thermal
Palakeeti et al. [24]	1 M NaOH at rt for 42 h. (**Imp-B**)	0.1M HCl at rt for 24 h. (**Degr-Imp-A1**) (**Degr-Imp-A2**)	(NS)	1% H_2O_2 for 24 h, rt. (**Degr-Imp-O**) (**Imp-C**)	solid drug, 105 °C for 10 days. (NDP)	1.2 million lux hr (vis) on a thin layer of drug for 11 days. (NDP)	(NS)
Suchitra et al. [25]	0.500 mg/mL rucaparib solution - 1M NaOH at 60°C for 30 min. (NSE)	0.500 mg/mL rucaparib solution - 1M HCl at 60°C for 30 min. (NSE)	(NS)	0.500 mg/mL rucaparib solution - 10% H_2O_2 at 60°C for 30 min. (NSE)	(NS)	1 mg/mL RUC stock solution, UV-vis light for 1 day. (NSE)	1 mg/mL RUC stock solution, 60°C for 6 h (NSE)

*(NS):no-studied condition; (NDP): no degradants observed; (NSE): no structure elucidation; Imp-B: rucaparib impurity found after basic forced degradation; Dergr-Imp-A1 and Degr-Imp-A2: rucaparib DPs formed in acidic forced degradation; Degr-Imp-O: rucaparib DPs formed in oxidative forced degradation; Imp-C: rucaparib impurity found after oxidative forced degradation.

Table S5. Collection of Area % values of olaparib, rucaparib, niraparib and corresponding DPs in the forced conditions studied.

Compound	RT (min)	B_MS area %	B_UV area %	A_MS area %	A_UV area %	N_MS area %	N_UV area %	O_MS area %	O_UV area %
Olaparib	6.89	100.0 - 47.2 (*) 100.0 - 24.4 (**) 100.0 - 0.7 (***)	100.0 - 56.2 (*) 100.0 - 18.3 (**) 100.0 - 1.0 (***)	100.0 - 99.7	100.0 - 101.2	100.0 - 102.0	100.0 - 103.3	100.0 - 101.1	100.0 - 99.9
DP-O1	6.23	0.0 - 10.4 (*) 0.0 - 13.1 (**) 0.0 - 25.9 (***)	0.0 - 32.4 (*) 0.0 - 34.3 (**) 0.0 - 75.0 (***)	(ND)	(ND)	(ND)	(ND)	(ND)	(ND)
DP-O2	4.25	0.0 - 94.7 (*) 0.8 - 78.5 (**) 1.2 - 65.7 (***)	0.0 - 51.2 (*) 0.5 - 40.7 (**) 0.8 - 37.9 (***)	0.0 - 19.6	0.0 - 6.8	(ND)	(ND)	(ND)	(ND)
DP-O4	6.62	(ND)	(ND)	(ND)	(ND)	(ND)	(ND)	0.4 - 1.1	0.5 - 1.4
Rucaparib	9.16	100.0 - 100.9	100.0 - 85.5	100.0 - 84.0	100.0 - 83.7	(NS)	(NS)	100.0 - 0.0	100.0 - 0.0
DP-R1	7.21	(ND)	(ND)	(ND)	(ND)	(NS)	(NS)	29.0 - 0.0	5.1 - 0.0
DP-R2	7.40	(ND)	(ND)	(ND)	(ND)	(NS)	(NS)	2.2 - 26.6	0.6 - 4.9
DP-R2'	7.33	(ND)	(ND)	(ND)	(ND)	(NS)	(NS)	0.0 - 0.0	0.0 - 0.0
DP-R3	6.90	(ND)	(ND)	(ND)	(ND)	(NS)	(NS)	7.0 - 106.8	0.6 - 0.8
DP-R3'	6.80	(ND)	(ND)	(ND)	(ND)	(NS)	(NS)	0.0 - 60.4	0.0 - 0.5
DP-R4	8.48	(ND)	(ND)	(ND)	(ND)	(NS)	(NS)	0.0 - 88.5	0.0 - 5.6
Niraparib	5.49	100.0 - 115.6	100.0 - 120.6	100.0 - 87.8	100.0 - 92.8	(NS)	(NS)	100.0 - 90.6	100.0 - 85.4

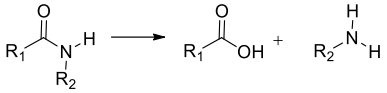
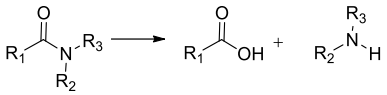
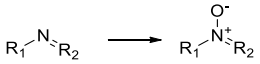
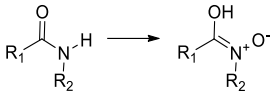
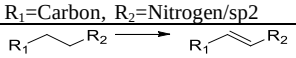
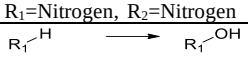
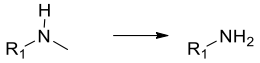
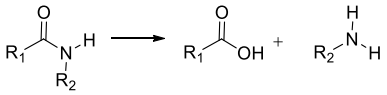
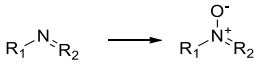
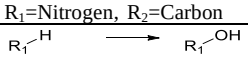
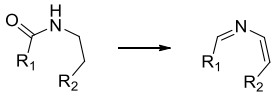
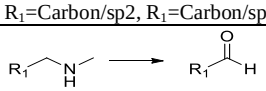
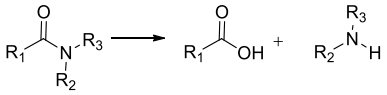
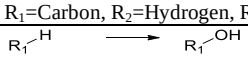
Values of MS area % and UV area % of the reported compounds at the beginning and at the final time of reactions. B: basic degradation; A: acidic degradation; N: neutral degradation; O: oxidative degradation; (*): values referred to the reaction performed in ACN:H_2O (25:75, v:v %); (**): values referred to the reaction performed in ACN:H_2O (12.5:87.5, v:v %); (***): values referred to the reaction performed in H_2O. (ND): compound not detected in the specified condition; (NS): condition not studied. The reported values are the merge of three independent replicates.

Table S6. MassChemSite settings used in the present work.

Setting type	Setting name	Value
General	Mass Spectrometer	Agilent Q-TOF (all)
	Acquisition mode	Auto MS/MS | DDS (all)
	Use retention time range (min)	None (all)
	Internal Standard	None (all)
	AutoSelect structures	True (all)
	Discard Structures with score below	0 (all)
	Exclude products without formula	False (all)
	Exclude products having an absolute *m/z* diff (ppm) above	False (all)
Analog Signals	Use UV peak area. Threshold (%)	0.20 (all)
	Use preset UV wavelength (nm)	254 (all)
	Wavelength selection method	None (all)
	Retention time delay (min)	Automatic (all)
	Radiolabeled	False (all)
	Fluorescence	False (all)
Products Generation	Minimum mass	50 (all)
	Ignore products stereochemistry	True (all)
	Ignore redundant products	True (all)
	MIM (%)	30 (all)
	Reactions	See Table S7
	Number of product generations	2 (O, N); 3 (R)
	Use constrained depiction	False (all)
Peak detection	Use maximum product count. Limit:	False (all)
	Area threshold	0.50 (%) 0 (absolute) (all)
	Peak detection smoothing	MEDIUM (O, N); NONE (R)
	Split computed peaks	False (all)
	Rescue computed peaks	False (all)
	Isotopes	MonoIsotopic Mass (MiM) (all)
	Filtering	Skip
	Adducts	None (all)
	Neutral Losses	False (all)
	Multiple-Charge Ions	True, max z = 2 (all)
	Dimeric Ions	False (all)
	Unexpected Products	True (all)
	Sum Areas	False (all)
Product Id	Reactant bond breaking limit	3 (O, N); 4 (R)
	Break products limit	2 (O, N); 3 (R)
	Exclude fragments having an absolute *m/z* diff (ppm) above:	10 ppm (all)
	Break 6-membered heteroaromatic rings	False (all)
	Oxidation during MS/MS	False (all)
	Bond breaking organizations	Even Electron, Odd Electron, N-Oxide (only MS) (all)
	Bond breaking experimental reorganizations	False (all)
MS Options	Same peak tolerance (amu)	0.010 (all)
	Chromatogram automatic filtering threshold	0.97 (all)
	MS automatic filtering threshold	0.95 (all)
	MS/MS automatic filtering threshold	0.95 (all)
	Ionization Mode	Positive (all)
	Spectra comparisons	2 (all)
	Signal Filtering	Automatic (all)
	Scan Filtering	Automatic (all)
	Saturation	False (all)

The differences between olaparib, rucaparib and niraparib are annotated with O, R and N, respectively. “all” is used when the specified setting is the same for the three drugs.

Table S7. Products generation reactions set in MassChemSite in this study.

Compd	Reaction name	MCS representation
olaparib	Amide (II) hydrolysis	 R_1=Carbon, R_2=Carbon
	Amide (III) hydrolysis	 R_1=Carbon, R_2=Carbon, R_3=Carbon
	N-oxide formation (1)	 R_1=Nitrogen, R_2=Carbon
	N-oxide formation (2)	 R_1=Carbon, R_2=Nitrogen/sp2
	Dehydrogenation	 R_1=Nitrogen, R_2=Nitrogen
	Hydroxylation	 R_1=Carbon
rucaparib	Amine (II) demethylation	 R_1=Carbon/sp3
	Amide (II) hydrolysis	 R_1=Carbon, R_2=Carbon
	N-oxide formation	 R_1=Nitrogen, R_2=Carbon
	Hydroxylation	 R_1=Carbon
	Degradation Imp-A2 formation	 R_1=Carbon/sp2, R_1=Carbon/sp2
	Imp-C formation	 R_1=Carbon/sp2
niraparib	Amide (I) hydrolysis	 R_1=Carbon, R_2=Hydrogen, R_3=Hydrogen
	Hydroxylation	 R_1=Carbon