Supporting Information

High-Throughput Screening Identifies Small-Molecule Enhancers of Reactive Oxygen Species that are Nontoxic or Cause Genotype-Selective Cell Death

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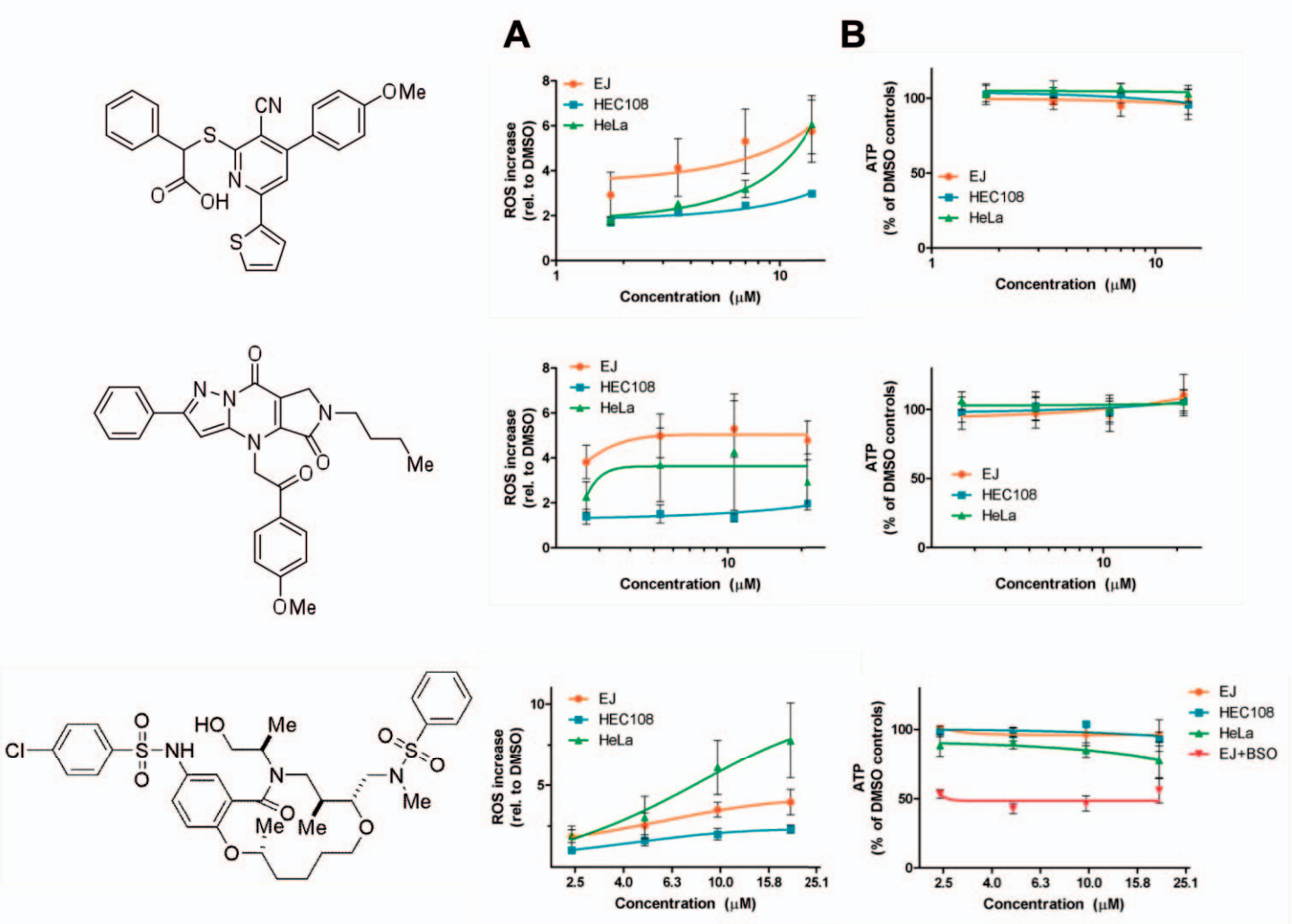
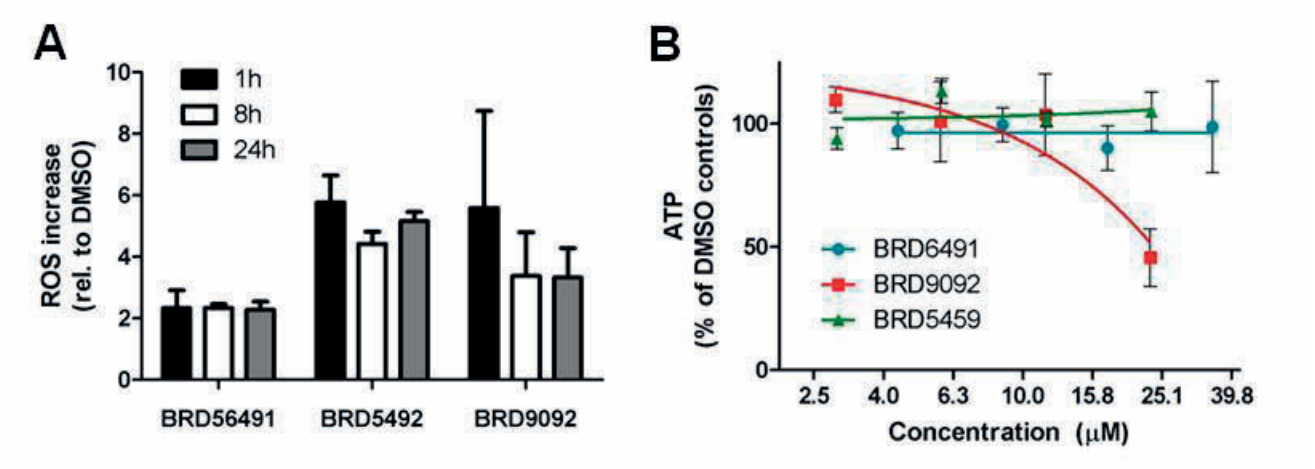
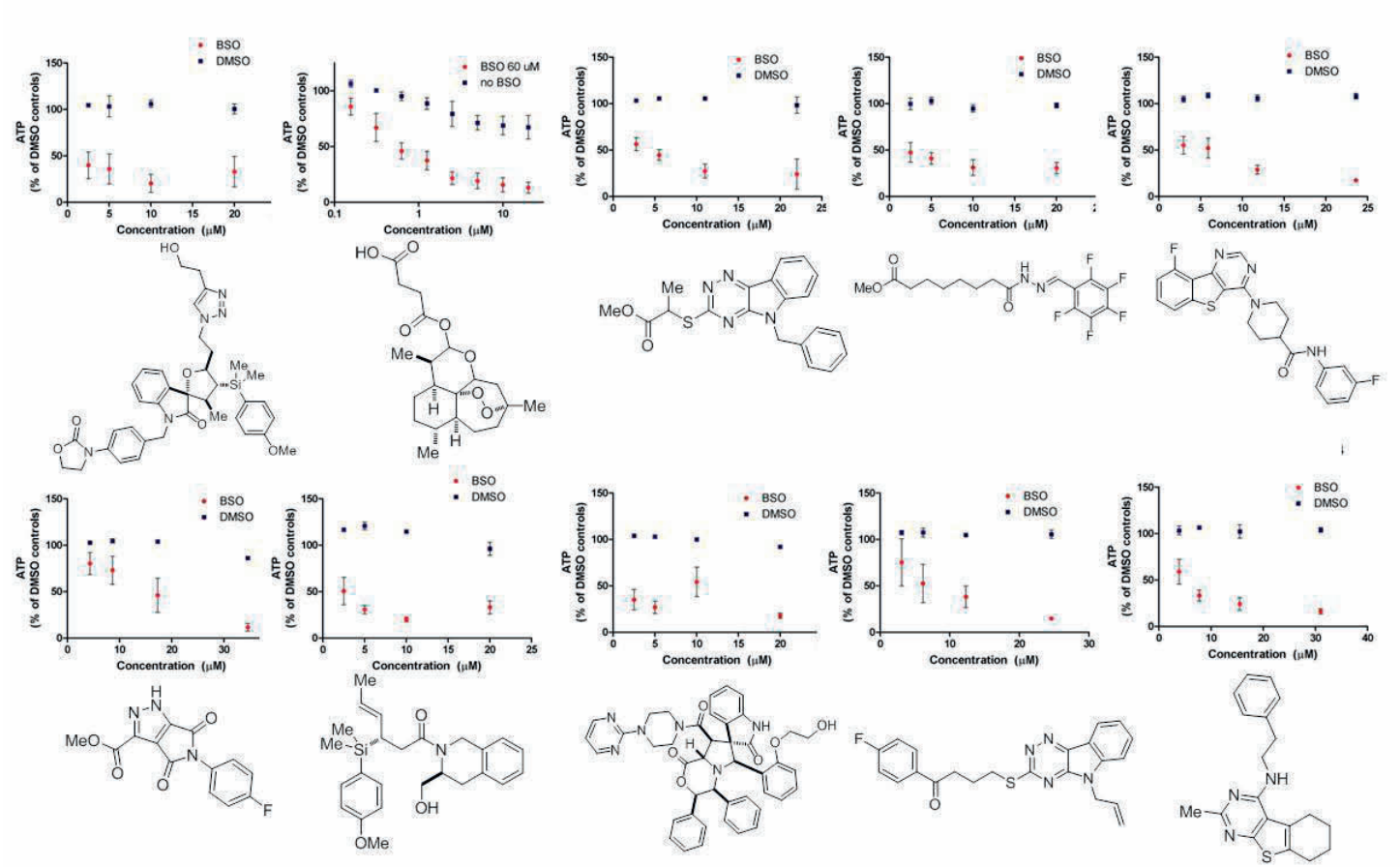


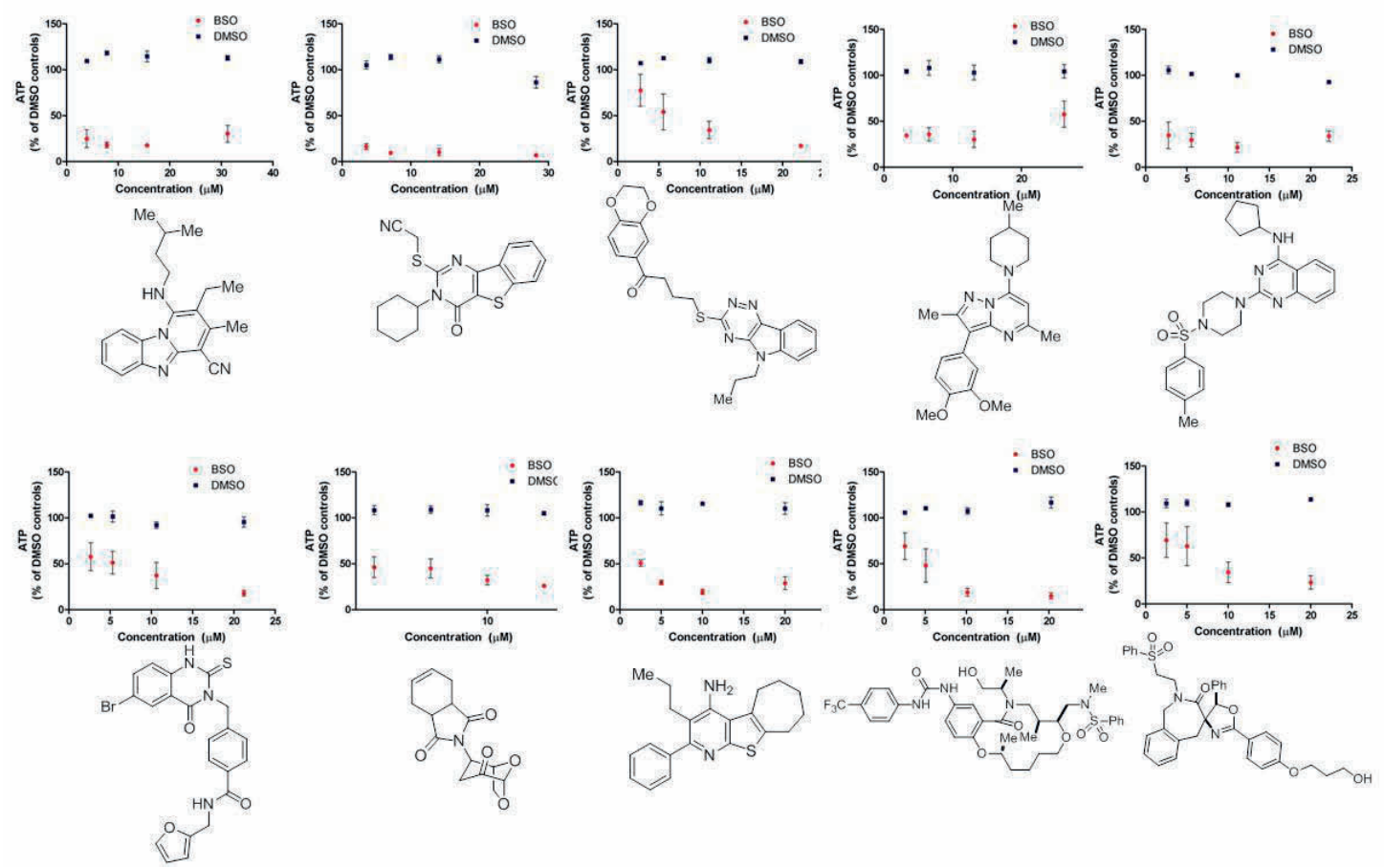
Supporting Figure 1. Three additional ROS-enhancing nontoxic compounds. A) Elevation of ROS for the indicated compounds after 1 hour in three cell lines. B) ATP levels after 48h in the same cell line panel. All data are expressed as mean±SD, n = 3.



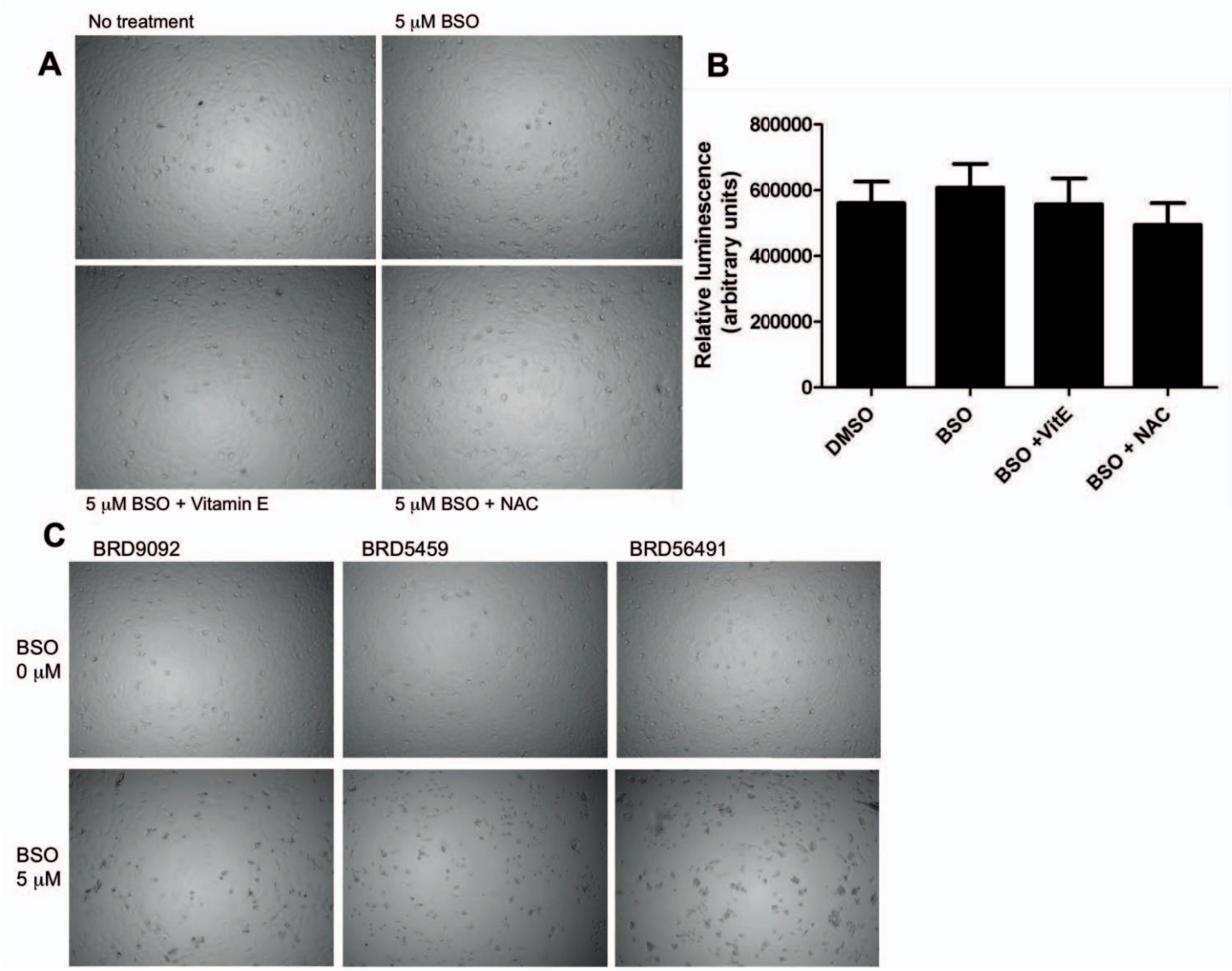
Supporting Figure 2. A) ROS levels were measured for 1, 8, and 24 hours in HeLa cells for three leading compounds, and normalized to DMSO treatment. B) Evaluation of cellular viability after 5-day treatment with the indicated concentrations of each compound in HEC108 cells. All data are expressed as mean±SD, n = 3.



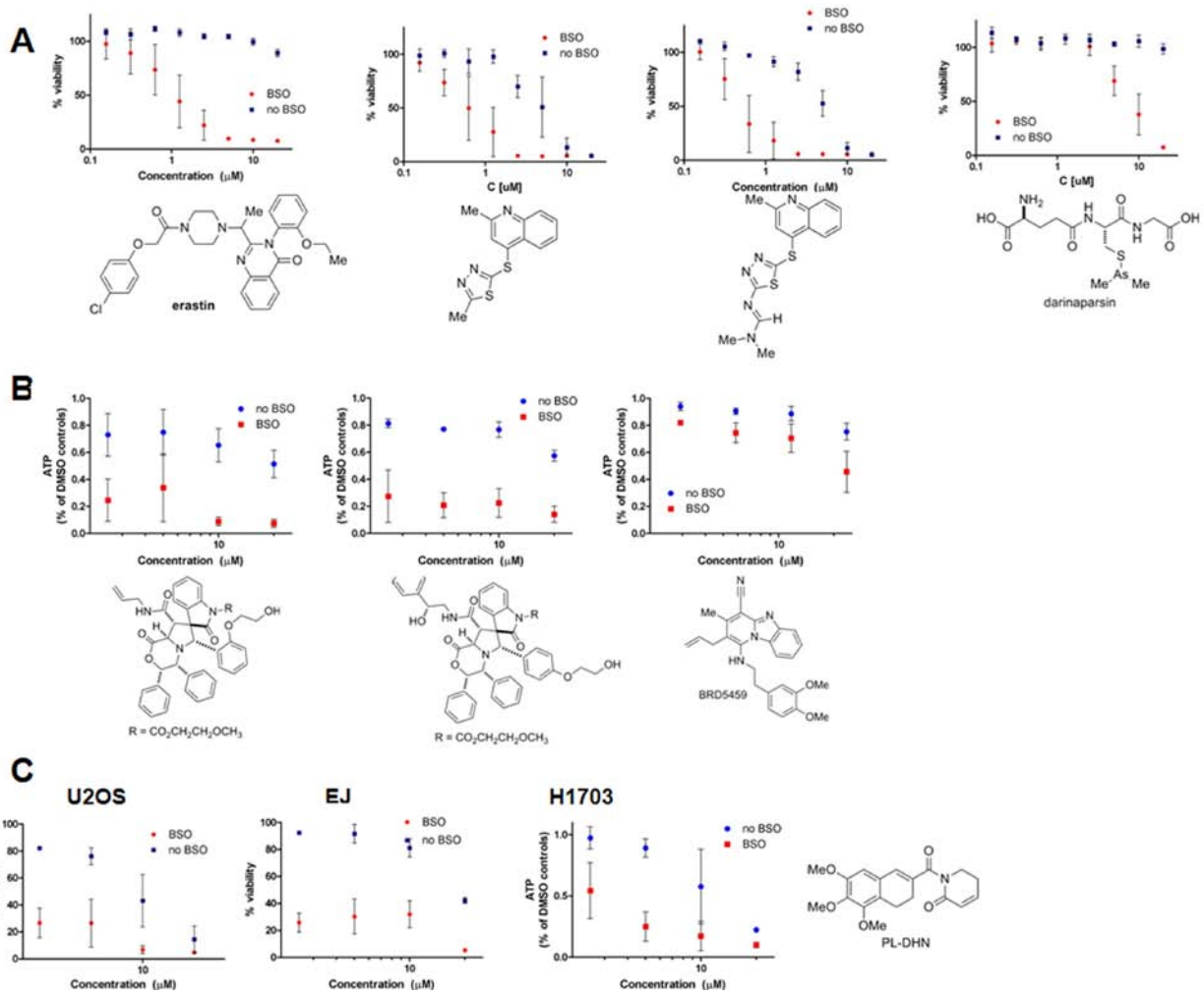
Supporting Figure 3A. Viability in EJ cells following 48-h treatment with ROS-enhancing small molecules only (blue) or co-treatment with nontoxic doses of BSO (red). All data are expressed as mean±SD, n = 3. Continues on next page.



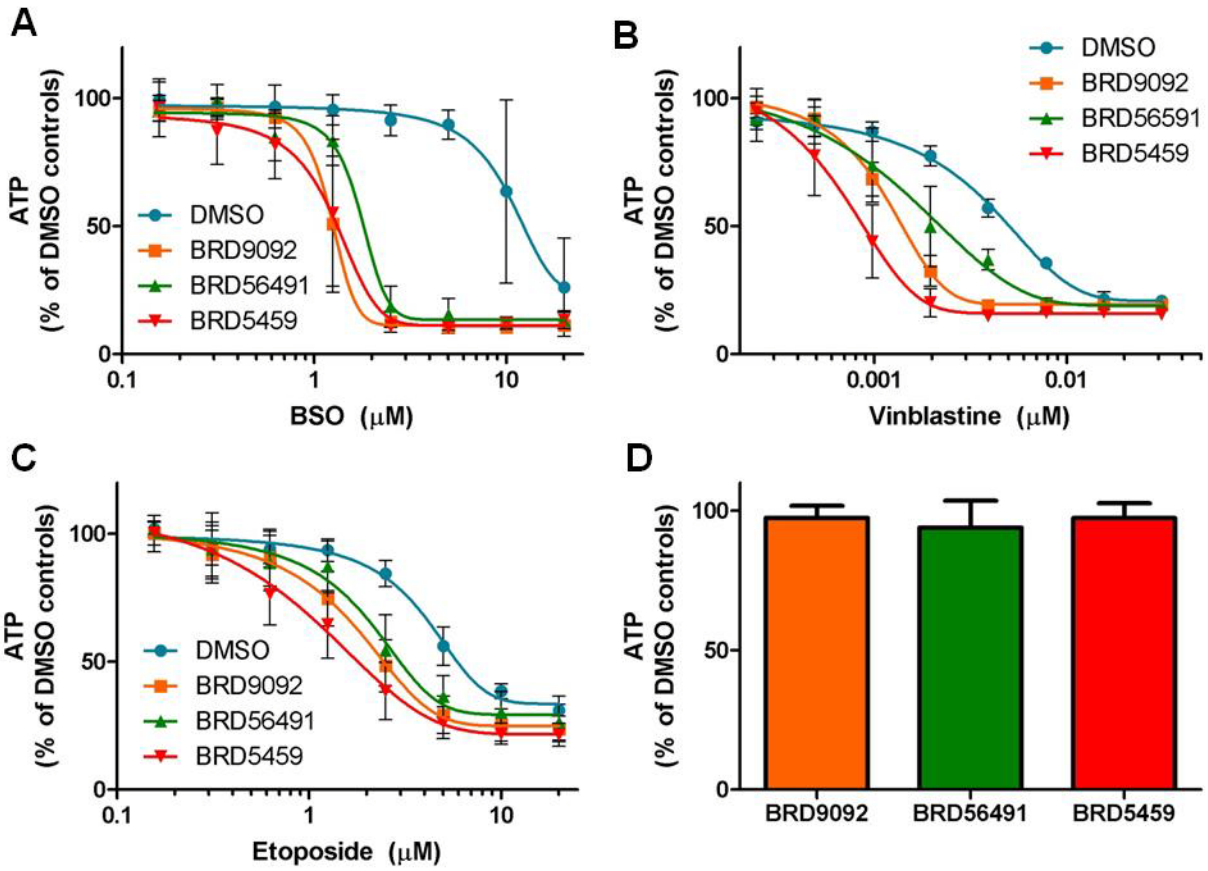
Supporting Figure 3B. Viability in EJ cells following 48-h treatment with ROS-enhancing small molecules only (blue) or co-treatment with nontoxic doses of BSO (red). All data are expressed as mean±SD, n = 3. Continued from previous page.



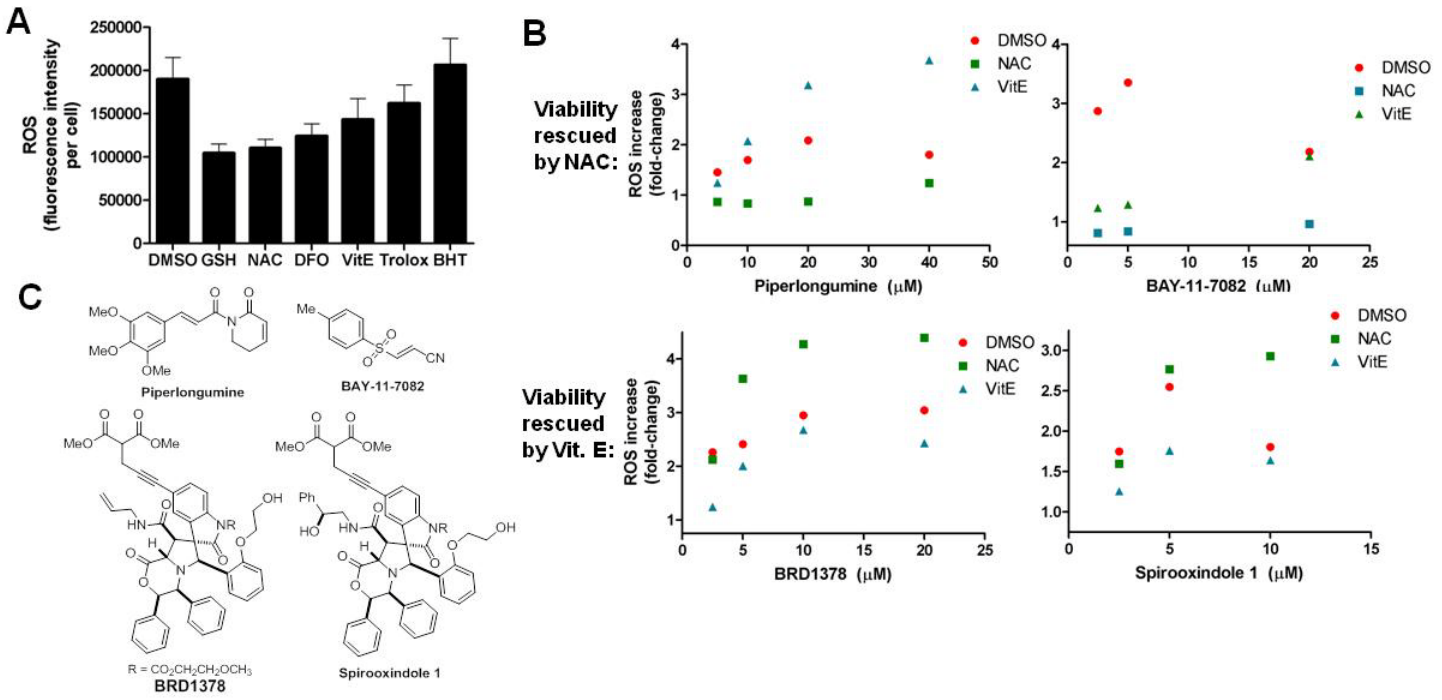
Supporting Figure 4. A) Bright-field microscopy images following 48-h BSO treatment with or without chemical antioxidants. B) For the conditions shown in A, ATP levels were obtained after 48-h treatment. Data are presented as mean±SD for four technical replicates and are representative of three independent experiments. C) Bright-field microscopy images following 48-h treatment with nontoxic ROS-enhancing compound treatments alone (upper), or co-treatment with BSO (lower). Morphological changes indicative of cell death are observed with BSO co-treatment. All experiments were performed in EJ cells.



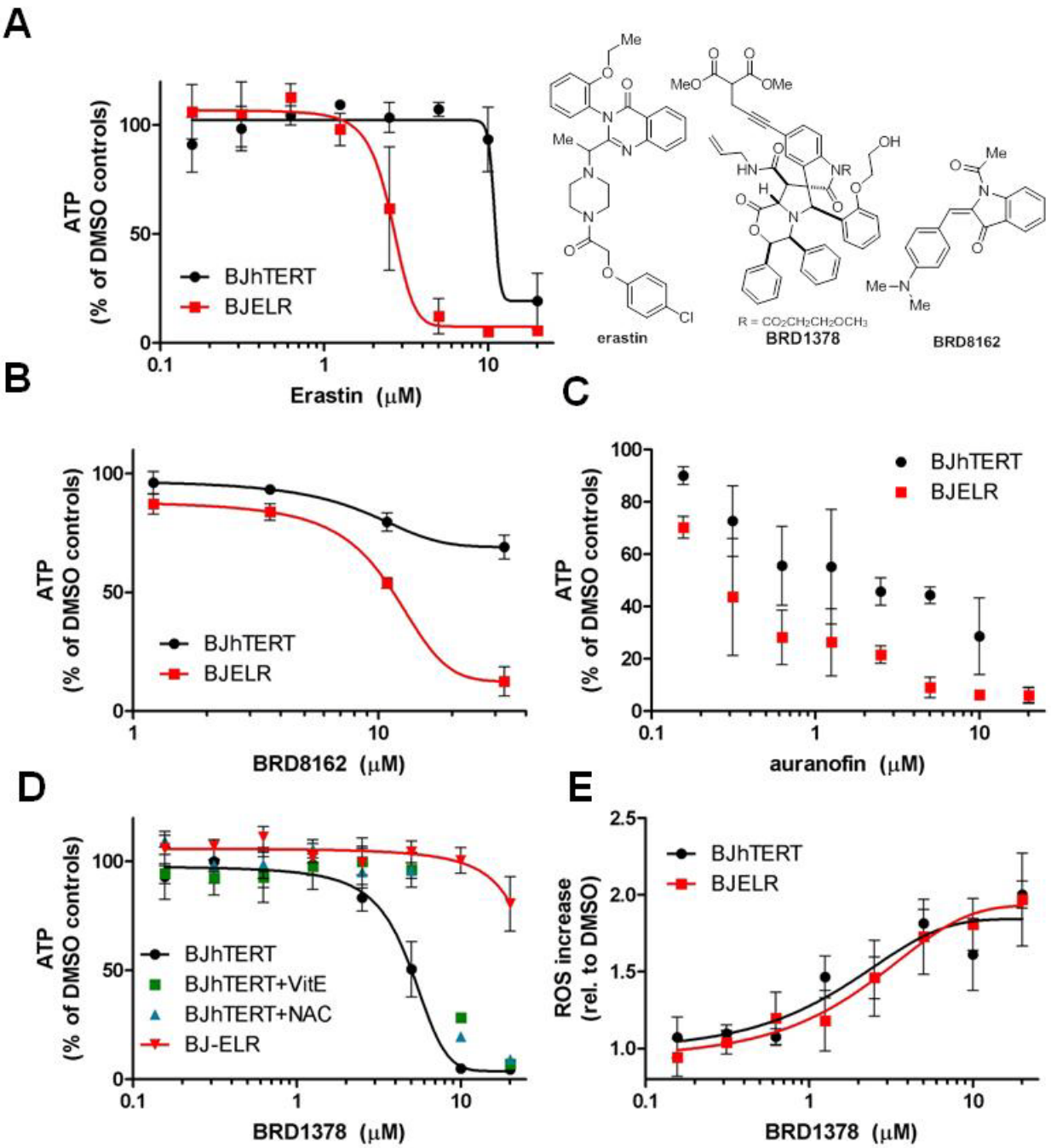
Supporting Figure 5. A) In U2OS cells, a distinct and in some cases structurally-related set of ROS-enhancing small molecules show strongly enhanced potency for reduction of cellular ATP levels when co-treated with a nontoxic dose of BSO (30 μM). **B)** In H1703 cells, a distinct and in some cases structurally-related set of ROS-enhancing small molecules show strongly enhanced potency for reduction of cellular ATP levels when co-treated with a nontoxic dose of BSO (10 μM). **C)** PL-DHN, a piperlongumine analog previously shown to enhance ROS and deplete glutathione levels in analogy to piperlongumine but with reduced cell death, shows strongly reduced viability when co-treated with BSO in three cell lines (EJ 5 μM, U2OS 30 μM, H1703 10 μM). All data expressed as mean±SD, n = 3 except H1703 cells plus 10 μM BSO, n = 2.



Supporting Figure 6. ATP levels in EJ cells measured following 48-h treatment with the indicated compounds co-treated with varying concentrations of BSO (A), vinblastine (B), and etoposide (C). D) ATP levels measured following 48-h treatment with the indicated compounds alone. All data are expressed as mean±SD, n = 3



Supporting Figure 7. A) Antioxidants lower cellular ROS. EJ cells were treated for 2h and stained with CM-H_2DCF-DA. Data are expressed as mean±SD for at least 48 technical replicates and are representative of three independent experiments. **B)** For a subset of compounds for which NAC (top) or vitamin E (bottom) rescues viability, ROS levels were measured with and without antioxidant treatment and fold-change was calculated relative to antioxidant (or DMSO) alone. NAC suppresses ROS increase for piperlongumine and BAY-11-7082, while vitamin E mitigates the increases observed for BRD1378 and Spirooxindole 1. Data are expressed as mean of two technical replicates and are representative of three independent experiments. **C)** Compounds employed in panel B).



Supporting Figure 8. ATP levels measured in BJhTERT and BJELR cells after 48-h treatment with erastin (A) and several electrophilic small molecules (B-C). All data are expressed as mean±SD, n = 3. D) Measurement of ATP levels in BJhTERT and BJELR after 48-h treatment with BRD1378. E) ROS increase measured in BJhTERT and BJELR following 1-h BRD1378 treatment. In contrast to the observed selective toxicity, ROS increase is similar in BJhTERT and BJELR. All data are expressed as mean±SD, n = 3.

Compound Structure	NAC		GSH		vit. E		Trolox		DFO		BHT	
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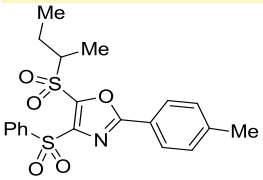
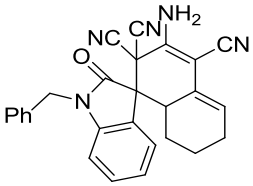
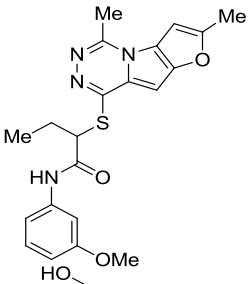
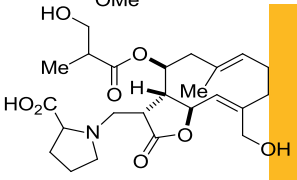
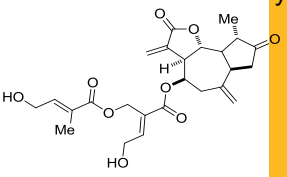
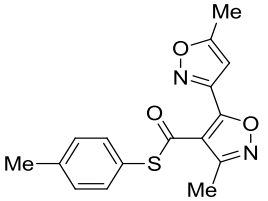
	yes	yes		yes								
		yes										
		yes										
	yes	yes										
	yes	yes	yes	yes								
	yes		yes									

Compound Structure	NAC		GSH		vit. E		Trolox		DFO		BHT	
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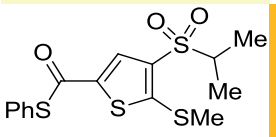
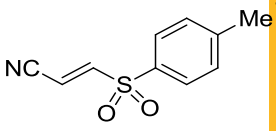
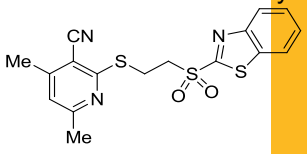
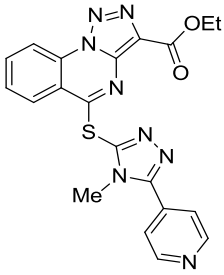
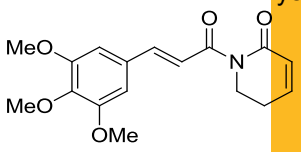
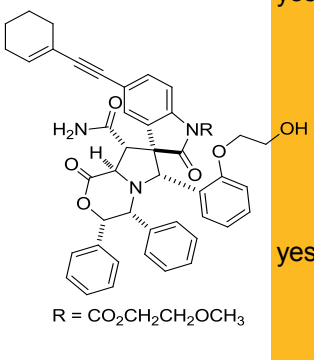
	yes	yes	yes	yes								
	yes	yes	yes	yes			yes					
	yes	yes	yes	yes								
	yes		yes									
	yes	yes	yes	yes								
 R = $CO_2CH_2CH_2OCH_3$	yes		yes									

Compound Structure	NAC		GSH		vit. E		Trolox		DFO		BHT	
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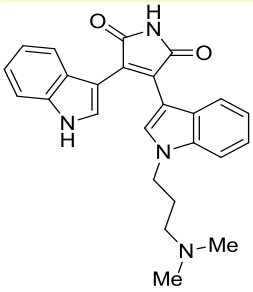
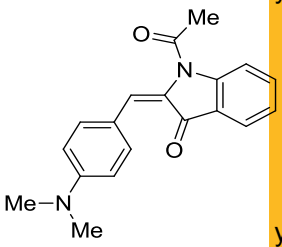
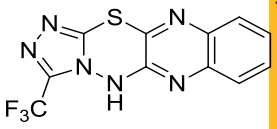
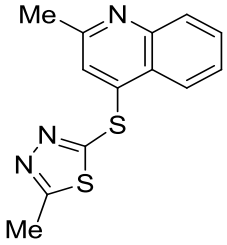
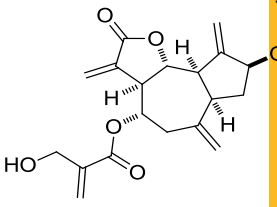
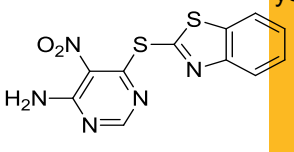
	yes											
	yes	yes										
	yes	yes	yes	yes								
	yes	yes	yes	yes					yes			
	yes	yes	yes	yes								
	yes	yes	yes	yes					yes	yes		

Compound Structure	NAC		GSH		vit. E		Trolox		DFO		BHT	
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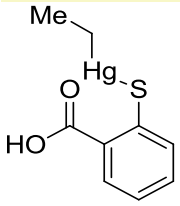
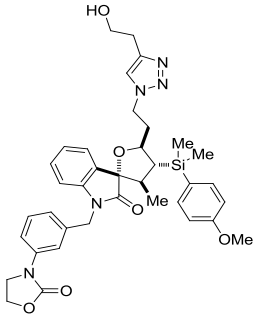
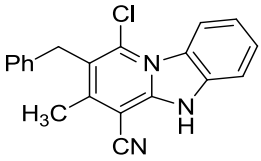
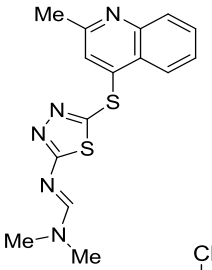
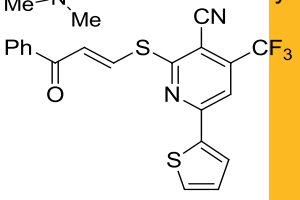
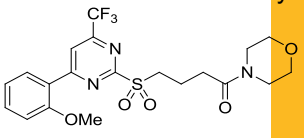
	yes	yes	yes	yes						yes		
		yes										
	yes	yes	yes	yes								
	yes	yes	yes	yes								
	yes	yes	yes									
	yes	yes	yes	yes								

Compound Structure	NAC		GSH		vit. E		Trolox		DFO		BHT	
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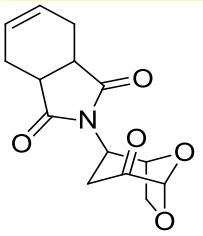
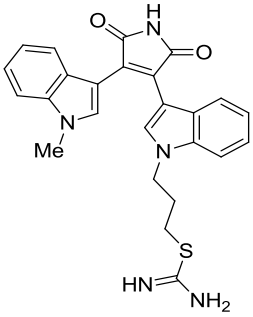
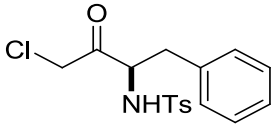
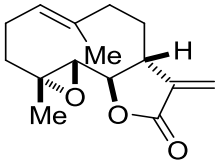
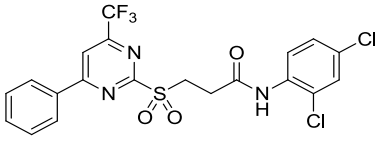
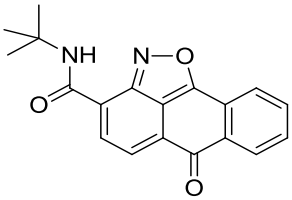
			yes									
			yes	yes								
			yes	yes								
			yes	yes								
												
				yes								
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Compound Structure	NAC		GSH		vit. E		Trolox		DFO		BHT	
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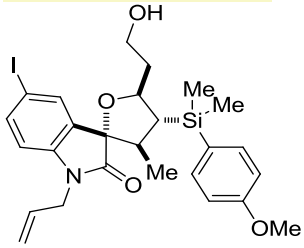
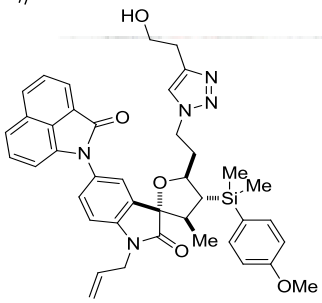
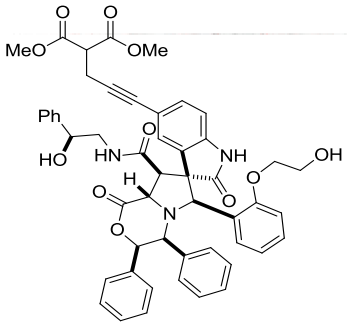
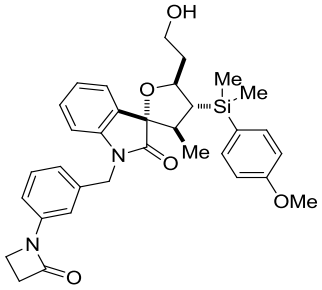
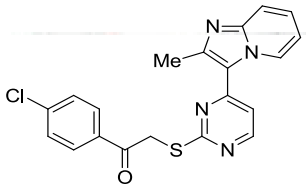
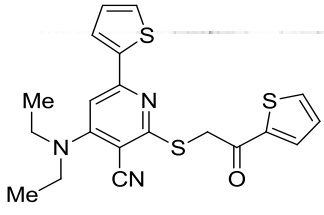
					yes	yes						
						yes						
					yes				yes			
						yes						
						yes						
					yes							

Compound Structure	NAC		GSH		vit. E		Trolox		DFO		BHT	
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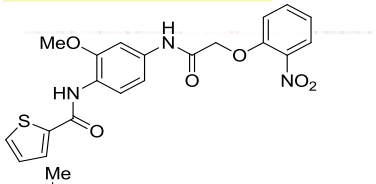
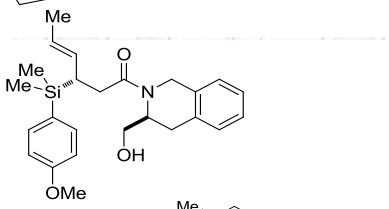
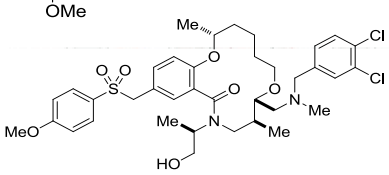
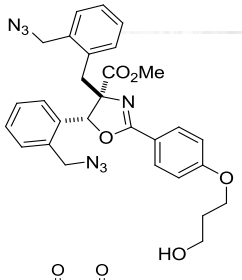
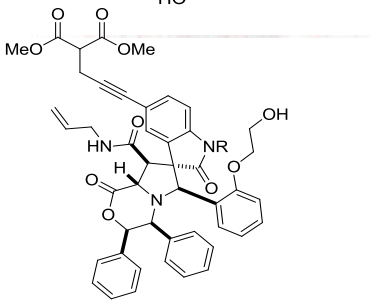
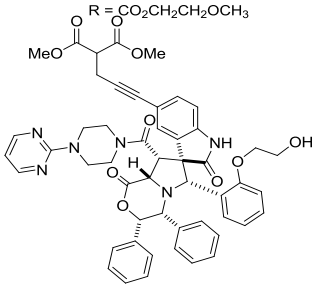
						yes						
						yes						
						yes						
						yes						
			yes									yes
							yes		yes			