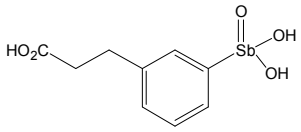
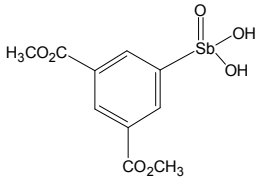
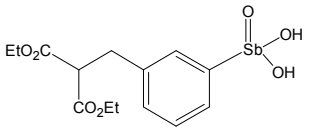
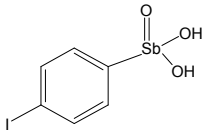
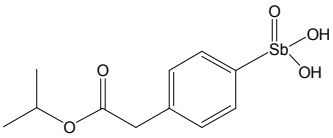
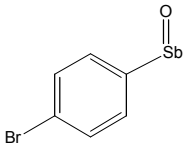
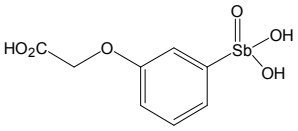
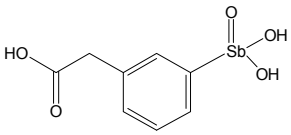
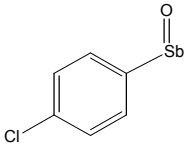
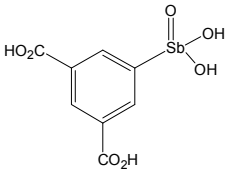
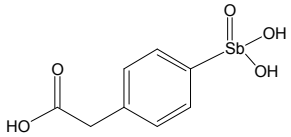
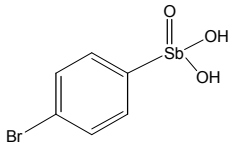
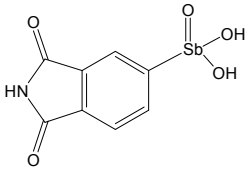
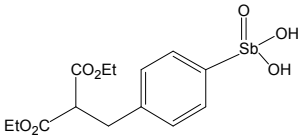
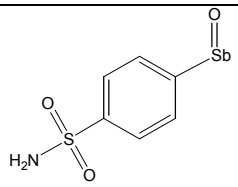
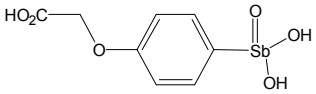
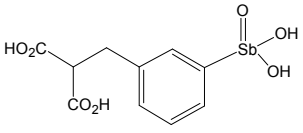
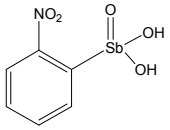
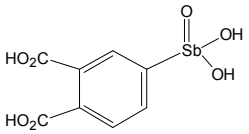
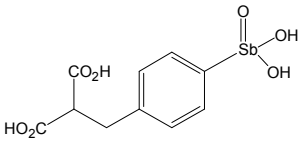
Supplemental Materials and Methods

Knockdown of Ape1 expression using siRNA. Modified Eagles Medium (MEM, Mediatech) supplemented with 10% fetal bovine serum (Hyclone) and penicillin/streptomycin (Invitrogen) was used for all tissue culture. The Ape1 siRNA (UCUGGUACGACUGGAGUACC) was purchased from Dharmacon. Antibodies were purchased from Imgenex. Human osteosarcoma (HOS) cells (American Type Culture Collection) were plated onto 6-well tissue culture plates at a density of 1.2 x 10^5 cells/well and allowed to adhere overnight. The following day, transfection of the siRNA into the cells was carried out using the standard procedure for Oligofectamine transfection reagent (Invitrogen). On the third day, the old media was removed and 0 or 100 μM MMS was added to the cells. After 48 h additional incubation, the cells were counted, diluted, and plated in triplicate onto 6-well tissue culture plates. Any remaining cells were rinsed once with $PBS^{-/-}$ and resuspended in RIPA buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1% NP-40, 0.5% deoxycholate Na^+, 0.1% SDS) for western analysis. Colonies were allowed to grow for one week prior to being rinsed with $PBS^{-/-}$ and fixed and stained with 6% glutaraldehyde and 0.5% crystal violet for 1.5 h. Stained colonies were counted and clonogenic survival was determined relative to untreated cells.

UNG activity assay. The UNG activity was performed using a uracil-containing molecular beacon hairpin substrate essentially as described (Jiang, et al., 2005). Reactions were carried out in the presence of 10-fold dilutions of **13778** and 100 pM human UNG.

Supplemental Table 1. Chemical Structures of the Stibonic Acid Sublibrary.

 **P6949**	 **P6967**	 **P6983**
 **P6952**	 **P6968**	 **13730**
 **P6953**	 **P6970**	 **13731**
 **P6954**	 **P6971**	 **13740**
 **P6964**	 **P6972**	 **13741**
 **P6965**	 **P6981**	 **13742**
 **P6966**	 **P6982**	

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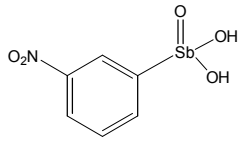
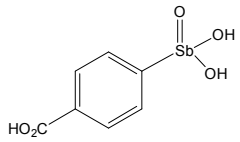
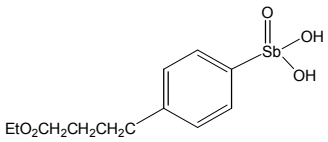
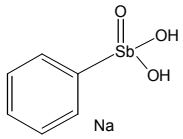
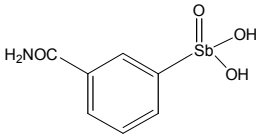
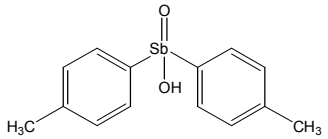
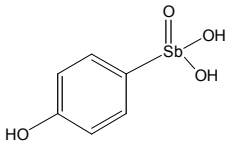
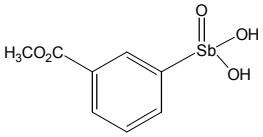
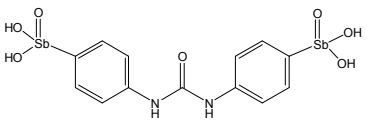
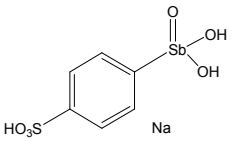
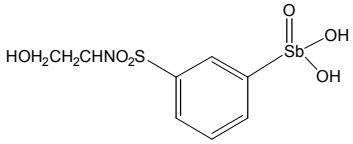
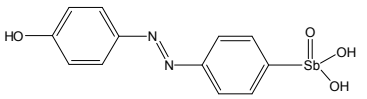
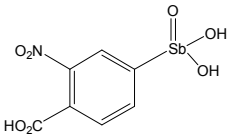
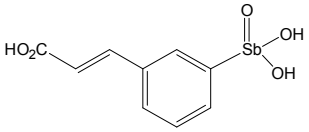
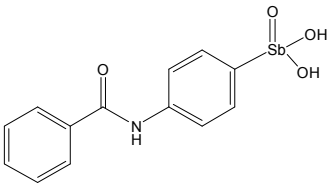
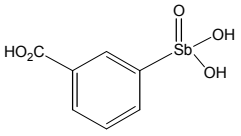
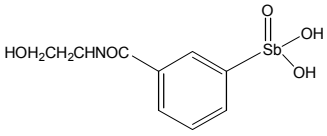
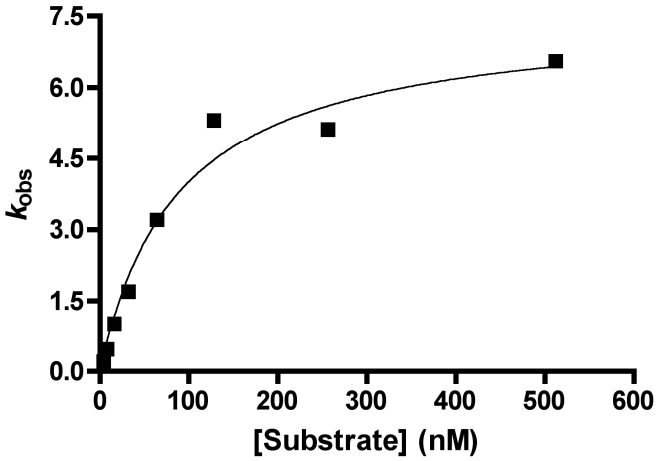
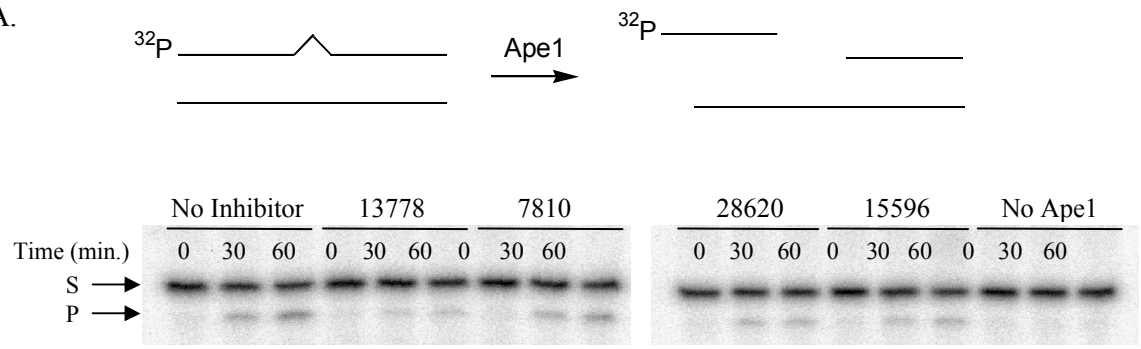
 **13743**	 **13760**	 **13793**
 **13744**	 **13765**	 **13794**
 **13745**	 **13771**	 **15408**
 **13746**	 **13776**	 **15591**
 **13755**	 **13778**	 **15596**
 **13759**	 **13782**	

Supplemental Figure 1: Kinetic analysis of Ape1 cleavage of the molecular beacon substrate. Reactions were carried out using increasing concentrations of the molecular beacon substrate and 5 pM Ape1 in the presence of 50 mM NaCl. Initial rate measurements were used to plot the Michaelis-Menten binding curve (shown) and determine the kinetic constants for the Ape1 molecular beacon substrate (k_{cat} = 8 ± 1 s^{-1}, *K*m = 90 ± 30 nM).



Supplemental Figure 2. Verification of Ape1 inhibitors using a secondary radioactivity assay. (A) The THF-containing Ape1 substrate was labeled on the 5' end with ^{32}P. The short DNA fragment produced by Ape1 cleavage (P) runs faster than uncleaved substrate (S) on a denaturing polyacrylamide gel. (B) Inhibitory activities of the four best Diversity Set hits were verified using the radioactive assay.

A.

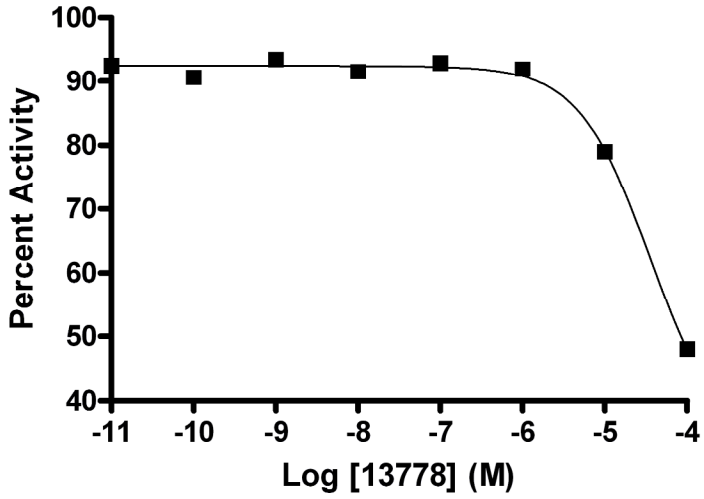


B.

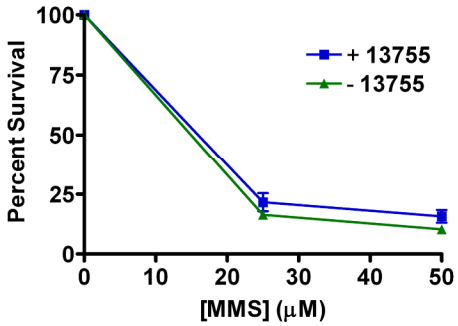
Compound	Percent Inhibition[a] (Radioactive Assay)
13778	81
P7810	35
15596	58
28620	63

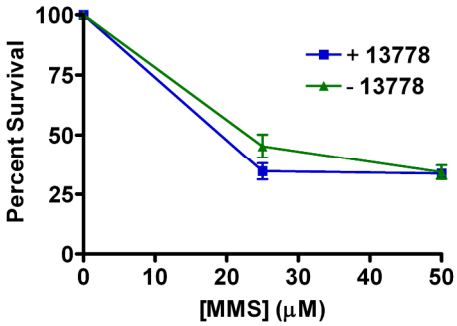
[a] Reactions were carried out in the presence of 50 nM substrate.

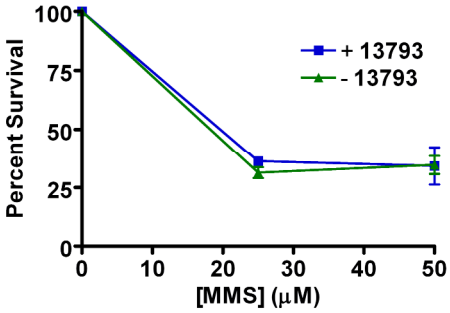
Supplemental Figure 3: Weak inhibitory activity of 13778 against human uracil DNA glycosylase (UNG). 13778 inhibits hUNG activity by 50% at a concentration of about 100 μM, which is over 300 times the IC_{50} of this compound for Ape1.

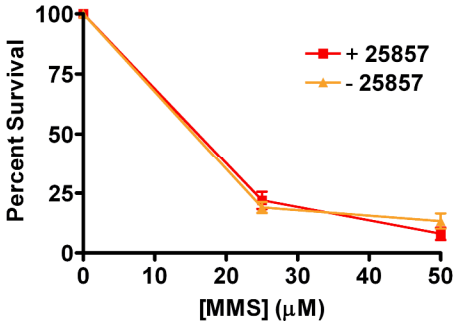


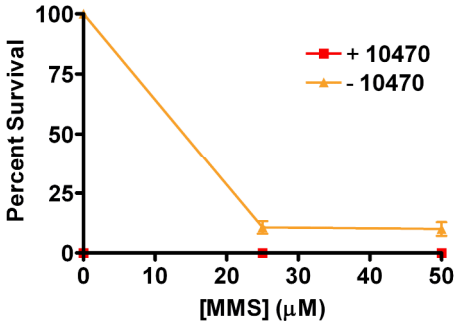
Supplemental Figure 4: Effects of selected Ape1 inhibitors on cell survival in the presence of MMS. Toxicity of MMS at varying concentrations was assessed in the presence and absence of 5 μM of the indicated Ape1 inhibitors. Compound **10470** was generally toxic at a 5 μM concentration (lower right panel).



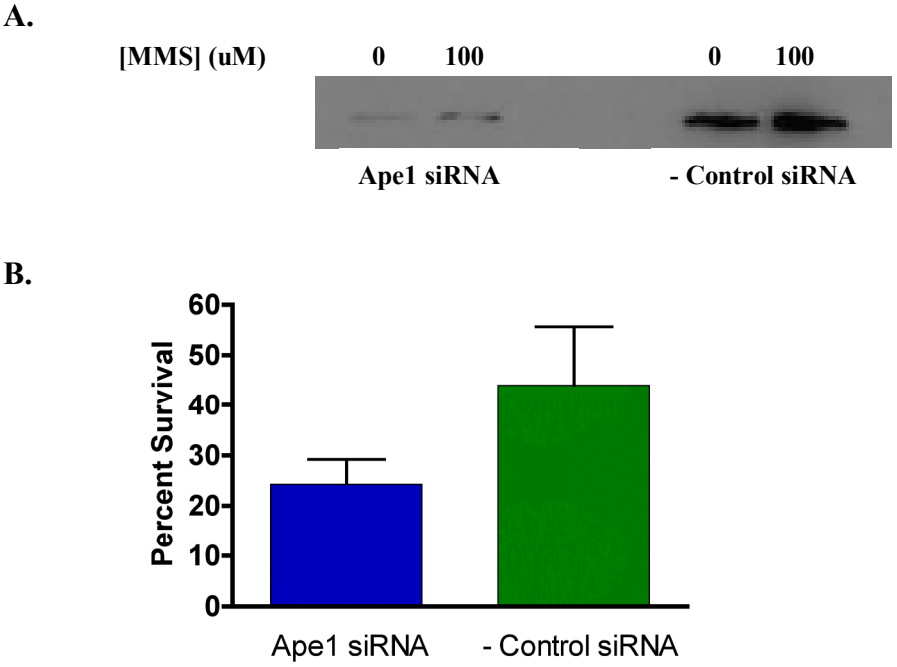








Supplemental Figure 5: Effect of siRNA knock down of Ape1 on the survival of HOS cells in the presence of MMS. (A) Ape1 protein levels (as measured by western blotting) were knocked down >80% using an Ape1 siRNA. (B) An ~2-fold decrease in cell survival was observed upon siRNA knock down of Ape1 in the presence of 100 μM MMS. Cell survival was unaffected by Ape 1 knockdown in the absence of MMS (not shown). The experiment was performed in triplicate for estimation of errors.



Supplemental References

Jiang YL, Krosky D, Seiple, L and Stivers JT (2005) Uracil-directed ligand tethering: an effective strategy for uracil DNA glycosylase (UNG) inhibitor development. *J Am Chem Soc* **127**:17412-17420.