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Evaluation of Novel Imidazotetrazine Analogues Designed to Overcome Temozolomide Resistance and Glioblastoma Regrowth

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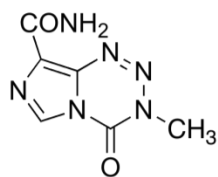
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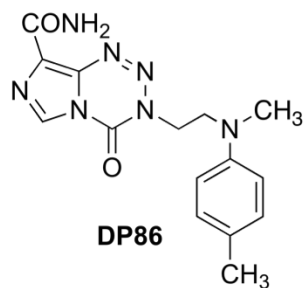
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Legends for Supplementary Figures for Ramirez et al.

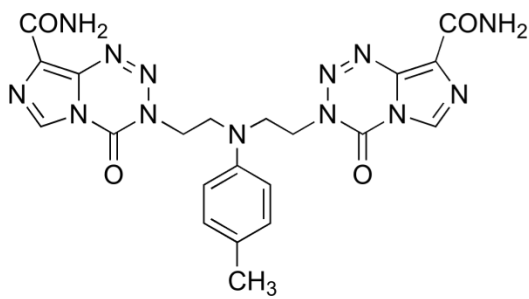
**Figure S1. Structures of TMZ, DP68, and DP86.**



**TMZ**



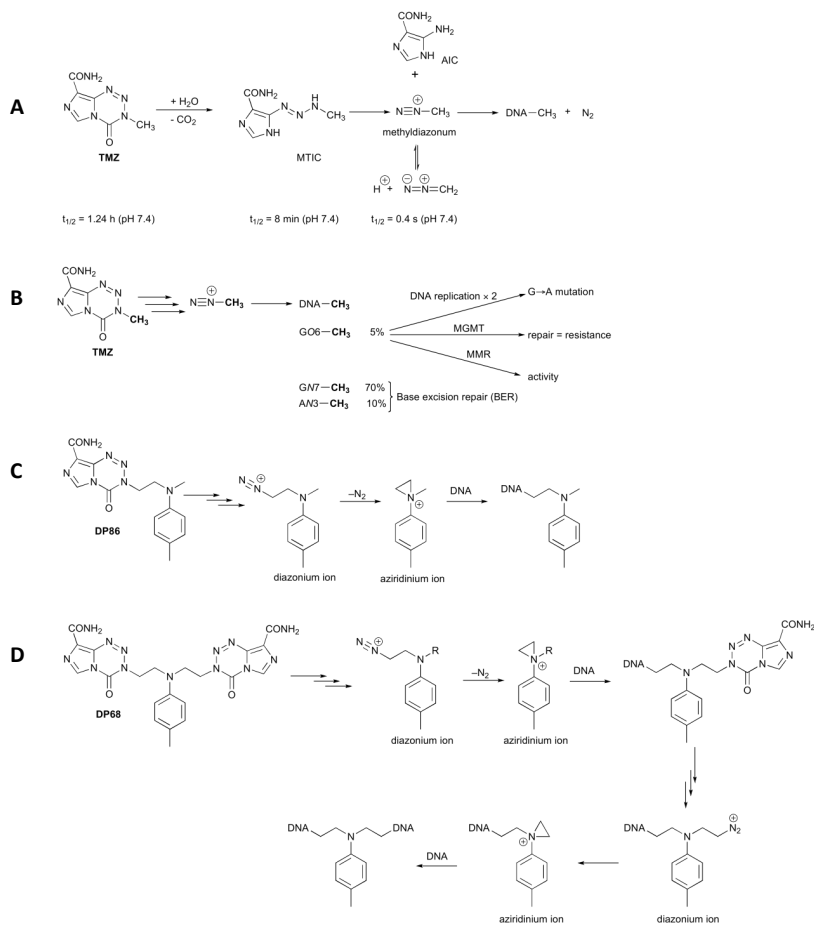
**DP86**



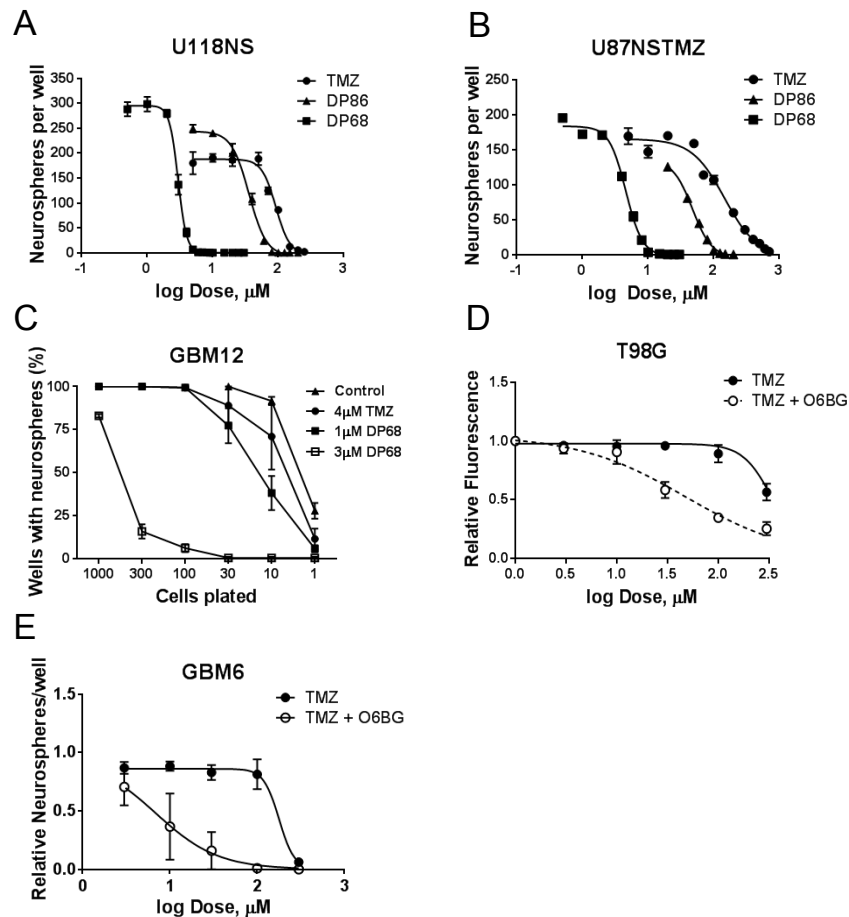
**DP68**

## Figure S2. Summary of prodrug activation.

Summary of prodrug activation and drug mechanisms of action for TMZ, DP68 and DP86 highlighting the roles of diazonium and aziridinium ion intermediates. (A-B) TMZ hydrolysis, generation of methyldiazonium ions and reaction with DNA; (C) DP86 monoalkylation; (D) DP68 crosslink formation.

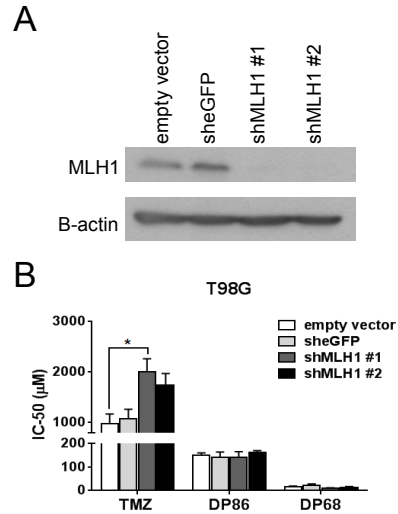


**Figure S3. MGMT sensitivity of anti-glioma activity of TMZ, DP86 and DP68.** (A-B) TMZ, DP68, and DP86 dose-response curves in (A) U118NS and (B) U87NSTMZ cell lines. All neurosphere lines were plated in triplicate and exposed to varying concentrations of each agent. Following a 7-day incubation neurospheres were quantified. Representative results from three independent experiments are shown. (C) GBM12 cells were plated with varying densities, treated with no drug (control), 1  $\mu$ M DP68, 3  $\mu$ M DP68 or 4  $\mu$ M TMZ. Neurospheres were counted after 14 days. (D-E) T98G (D) and GBM6 (E) cultures were co-treated with 0 or 10  $\mu$ M O6-BG and response to TMZ was evaluated. Cell survival was analyzed via CyQuant assay with the mean relative fluorescence  $\pm$  SEM from three independent experiments are shown.



**Figure S4. DP86 and DP68 activity is independent of MMR expression.**

(A) Western blot confirmed knockdown in T98G cells infected with empty vector, sheGFP, and two shRNAs targeting MLH1. (B) Infected cells were treated with TMZ, DP86, or DP68 and cell survival was analyzed via CyQuant assay. Mean  $IC_{50} \pm SEM$  from three independent experiments are graphed. \* ( $p < 0.05$ )



**Figure S5. DNA damage signaling activated by low concentrations of DP68.** U251 and T98G cells were exposed to DP68 for 24 hrs and whole cell and nuclear extracts were processed for Western blotting. Representative blots from three independent experiments are shown.

