3-Amino-1,2,4-benzotriazine di-N-oxide (tirapazamine, TPZ) belongs to a new class of anti-tumor drugs and requires bio-reductive activation for its medicinal properties. Tirapazamine derives anti-tumor properties from its ability to cause DNA damage in oxygen poor conditions found in solid tumors. However, the exact DNA damaging agent produced from activated TPZ is still a matter of debate.

In our studies, we investigated into the exact nature of DNA damaging agent(s) produced from activated TPZ and its related di-N-oxide compounds. As part of our mechanistic studies we carried out DNA cleavage assays, isotopic labeling studies and radical trapping experiments. Our data shows that TPZ and its related di-N-oxide compounds upon bio-reductive activation produce known DNA damaging agent hydroxyl radical. Thus, our results suggest that TPZ and its structurally related heterocyclic di-N-oxides deliver known radiotherapeutic DNA damaging agent hydroxyl radical especially in oxygen poor environment found in solid tumors.