

Metal Pro-Chelators as Therapeutic Agents

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Metal chelators have long been used to treat toxic accumulations in the body, and some work has been done to use chelators as anticancer agents by depriving cancerous cells of Fe and Cu to slow proliferation. Recent efforts in the field focus on the use of pro-chelators, which promise improved selectivity, and thus a reduction in off target side effects.

Metals such as Fe and Cu play an important role in many cellular functions, ranging from oxygen transport to a broad array of enzymatic catalysis. As such, their concentration and availability within the cellular and extracellular environment is tightly controlled. If a buildup of either ion occurs within the body, toxicity can be observed. Other metals such as Cd, Pb, and Hg can show toxic effects at much lower concentrations, as the body has no means of regulating their concentration. The use of metal chelating agents to treat toxic metal buildup began in 1945 with the use of 2,3 dimercaptopropanol (BAL) to treat acute arsenic poisoning from chemical weapons use in WWII¹. The use of BAL was subsequently expanded to treating lead poisoning. Since this time, a number of other agents with less side effects have been developed to treat buildups of other divalent metal ions²⁻⁴. Besides being used to treat metal accumulation, the chelating properties of various organic compounds have been used as anticancer agents, and have been observed to inhibit bacterial growth⁵⁻⁷. The common theme in these treatments is severe side effects due to non-specific metal chelation throughout the body. As such, current research in the field is focused on the synthesis of pro-chelators, which are in an inactive form until triggered by some stimulus to reveal a metal chelating moiety.

Franz et al. have reported a pro-chelator that releases pyrithione (PT) upon the action of β -lactamase⁸. Pyrithione had been shown previously to have antimicrobial effects, making it an ideal candidate for protection into a pro-chelator⁶. PcephPT (phenylacetamido-cephem-pyrithione) which releases pyrithione upon cleavage by β -lactamase was synthesized and studied (Figure 1).

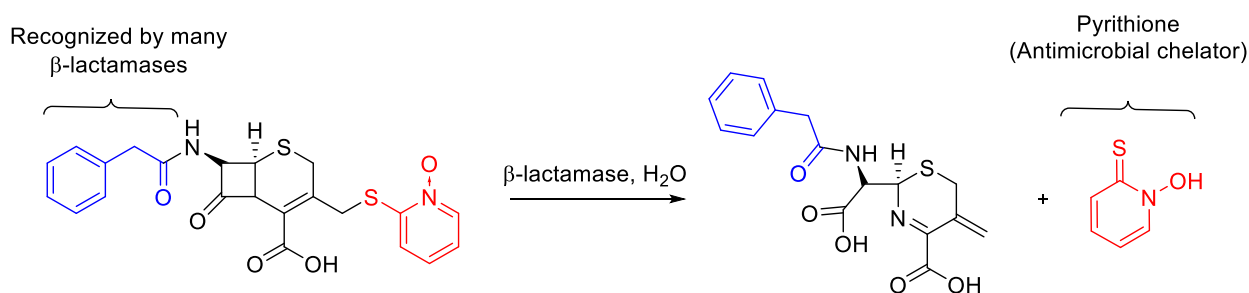


Figure 1: Approach to selectively target drug-resistant bacteria.

Solutions of PcephPT were found to be stable in cell media, but efficiently cleaved in the presence of β -lactamases capable of cleaving cephalosporins, releasing PT⁸. Cleaved PcephPT showed MIC values of 70 μ M or better. PcephPT showed significantly lower cytotoxicity in human cell cultures than PT, showing its improved specificity.

A hallmark of cancerous cells is rapid proliferation. Due to rapid growth, cancer cells have heightened needs for nearly every cellular resource, including Fe for use in enzymes such as ribonucleotide reductase, an enzyme critical for DNA synthesis. Chelation of intracellular Fe would thus cause cell cycle arrest, and eventually apoptosis. In fact, there have been a number of trials using thiosemicarbazones to take advantage of this strategy⁹. However, non-specific chelation has been shown to result in excess formation of methemoglobin in the body, leading to hypoxia⁹. As the off-target toxicity is only seen in the extracellular environment, selection of the appropriate intracellular chelate release trigger is expected to improve cancer specificity. Tomat et al. have shown that a redox sensitive disulfide bridge masked complex can successfully improve the specificity of thiosemicarbazones, and other similar compounds¹⁰ (Figure 1).

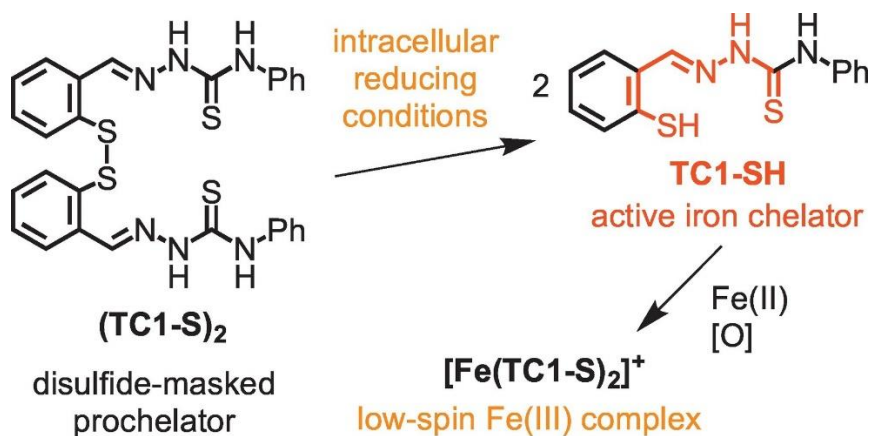


Figure 2: Diagram of pro-chelator design, release, and complex formation.

The intracellular environment has a much higher concentration of free thiols than the extracellular environment, able to trigger a redox sensitive species, such as a disulfide bond. It was shown that the ability to inhibit tumor growth was comparable to parent chelates, without inhibiting the ability of erythroid cells to replicate¹⁰. This result shows the utility of the pro-chelation strategy.

8-hydroxyquinoline (8-HQ) has been shown to have a wide range of bioactivities, including anti-leishmanial and anticancer effects, as a Cu^{2+} and Fe^{2+} chelator^{11,12}. The anticancer activity in particular makes 8-HQ an attractive area of research. However, as Cu and Fe are both important in a wide range of biological processes, it is desirable to limit the activity of 8-HQ to within

cancerous cells. Yin et al. have shown that the activity of 8-HQ can be regulated by addition of a phenylboronic acid protecting group, and enhanced by addition of a furoxan NO donor moiety¹³ (Figure 3).

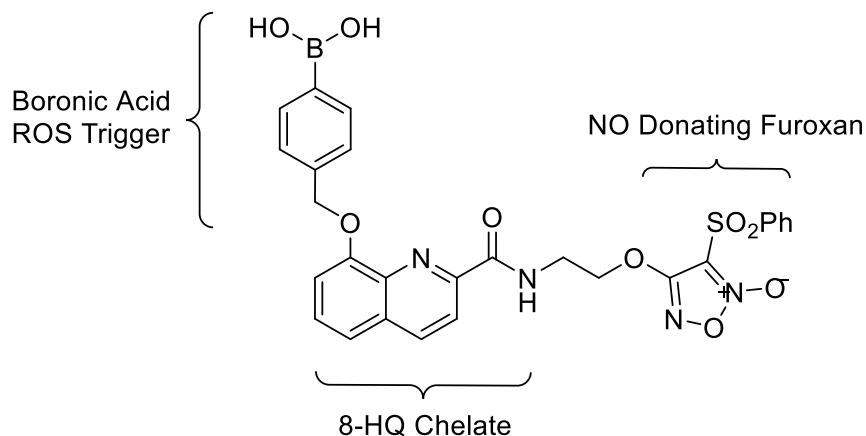


Figure 3: Structure and functionality of studied pro-chelator, HQ-NO-11.

Phenylboronic acid can be removed by reaction with reactive oxygen species (ROS), the levels of which are often elevated in cancer cells. The released chelate will then release its NO moiety, and form a Cu complex, the structure of which is unknown. However, it is known that the complex formed generates ROS, which causes cytotoxicity, while simultaneously promoting the deprotection of remaining pro-chelate¹³. Assays for cytotoxicity in healthy human cell lines showed low cytotoxicity, which allowed for *in vivo* trials using mice xenografts. Pro-chelators showed similar ability to inhibit tumor growth as the original 8-HQ chelate, and better than the negative control¹⁰.

The role of metal chelators in treating disease is largely limited by the desire to avoid widespread non-specific chelation of essential metal ions such as Fe and Cu. The creation of pro-chelators from the existing pool of metal chelates with known biological activity has the potential to greatly expand the role of metal chelation in the treatment of diseases ranging from bacterial infection to cancer.

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