Synthesis and characterization of DMT1 inhibitors

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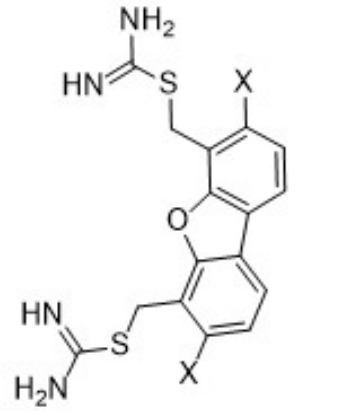
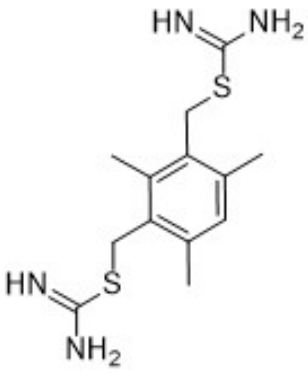
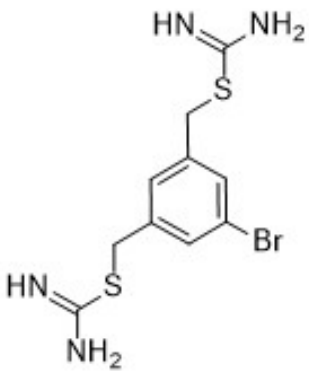
DMT1 is a proton coupled iron transporter which plays an essential role in iron homeostasis, and whose deregulation is linked to hemochromatosis. In the context of studying the structure and biological role of DMT1 in iron overload, we aim to obtain potent and selective small molecule inhibitor tool compounds. As a starting point we resynthesized the bis-cationic inhibitors **1, 2** and **3** previously reported by Cadieux *et al.*, [1], and confirmed their activity using both a radiolabeled iron uptake assay, although to a lower level than the reported values. We further confirmed their inhibitory activity using a fluorescence assay [2] as well as by electrophysiology in *Xenopus laevis* oocytes, and prepared heavy atom analogs such as the bromo-aromatic derivative **4** to assist structural studies. Inhibitory activity was further explored with purified prokaryotic DMT1 analogs [3] in proteoliposomes, and in ex-vivo intestinal iron uptake assay. These data show that these bis-cationic inhibitors represent a robust starting point to develop more potent analogs as DMT1 tool compounds.



1 (X = Br, IC_{50} = 1.52 µM *(lit.: 0.11 µM)*)
2 (X = H, IC_{50} = 1.83 µM *(lit.: 0.05 µM)*)



3 IC_{50} = 0.35 µM



4 IC_{50} = 4.66 µM

[1] Cadieux *et al, Bioorganic & Medicinal Chemistry Letters*, **2012,** *22*, 5108 – 5113.
[2] Montalbetti *et al*. *J Biomol. Screen.*, **2014**, *19,* 900 - 908**.**
[3] Ehrnstorfer *et al*., *Nat Struct Mol Biol*., **2014,** 21, 990 – 6. Ehrnstorfer *et al*., *Nature Communication,* **2017,** 8, 14033.