

## Design and Synthesis of Manganese based MRI contrast agent: A dual contrast under different MRI parameters

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**Introduction:** Application of Gadolinium (Gd) based MRI contrast agents is limited in patients with severe renal disease or following liver transplant due to association of a recently reported incurable and nonmitigable Nephrogenic Systemic Fibrosis (NSF) disease<sup>1</sup>. These limitations give rise to choose Mn<sup>2+</sup> (having 5 unpaired electrons) as a better paramagnetic label to obtain MRI contrast agents. As Manganese is a natural cellular constituent and often acts as a cofactor for enzymes and receptors. The capability of Mn<sup>2+</sup> to cross Blood Brain Barrier (BBB) provides a possibility of it to be used for brain imaging in case of neurological disorders. Mn<sup>2+</sup> is also not safe in its ionic form but can be utilized after coordinating with a suitable ligand leading to reduced toxicity. In these series only two agents, the liver-specific manganese (II)-dipyridoxal diphosphate (MnDPDP, Teslascan, Amersham Health) and an oral agent containing manganese (II) chloride (LumenHance) are available clinically for human use. Besides its T<sub>1</sub> shortening effect, Manganese also has a minor T<sub>2</sub> effect, which reduces the signal intensity to produce dark signals<sup>1</sup>. In the context of its dual property, we have designed and synthesized a new stable Manganese based MRI contrast with a capability of providing dual contrast in comparison to other conventional MRI contrast agents with a possibility of binding with Amyloid plaques in Alzheimer's disease<sup>2</sup>.

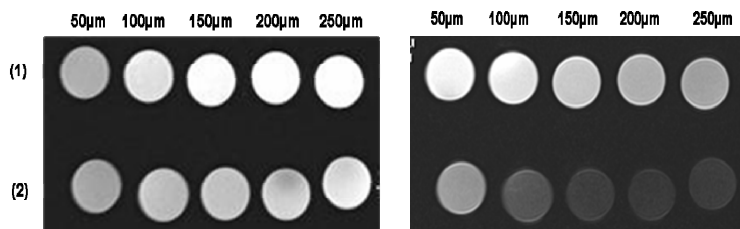
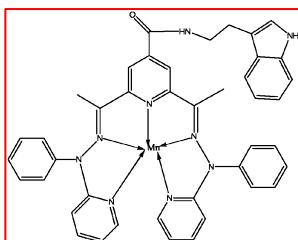
**Materials and Methods:** The ligand with five donor atoms has been synthesized using simple organic chemistry. Pyridine have been incorporated in the ligand due to their weak ligand nature providing high spin state (having five unpaired electrons) to the central metal ion (Mn<sup>2+</sup>) in the resulted MRI contrast agent. Step wise synthesis of the ligand and its characterization by NMR and IR spectroscopy was carried out. The final ligand was reacted with Manganese perchlorate in order to obtain a paramagnetic metal complex. The metal complex was then purified with the help of vapour diffusion crystallization process using Diethyl ether as a diffusion solvent. The pure crystals of the metal complexes were analyzed by IR spectroscopy in order to confirm the metal complexation reaction. The characterized Manganese complex was dissolved in 10% DMSO Solution (10%DMSO +90% H<sub>2</sub>O) due to hydrophobic nature of ligand. The phantoms were prepared with the solutions of varying concentrations of Manganese complex as well as with Gd-DTPA as a standard. The r<sub>1</sub> and r<sub>2</sub> relaxivities of the contrast agent have been determined on a Siemens Skyra Magnetom 3T MRI whole body scanner. T<sub>1</sub> relaxation times for the different concentrations of agents using series of standard Inversion Recovery pulse sequence with variable inversion times (TI) of 50, 150, 300, 500, 750, 1000, 1250, 1500, 1750, 2000, 2250, 2500 and 3000 ms with TR=3500ms and TE =14ms. The intensity values were utilized from the MRI images in order to evaluate T<sub>1</sub> values for every concentration. Spin-echo pulse sequence has been performed on the similar phantom with the echo times (TE) 16, 33, 49, 65, 81, 98, 114, 130, 146, 163, 179, 195 228 ms and TR=4810 ms for T<sub>2</sub> calculations. The T<sub>1</sub> and T<sub>2</sub> weighted images have been further acquired using spin echo pulse sequence with repetition times TR as 600ms, 4820ms and echo times TE as 11 and 228 ms respectively.

**Results and Discussion:** The lower two images are T<sub>1</sub> and T<sub>2</sub> weighted MRI images of phantoms containing varying concentrations from 50-250 μm of both the contrast agents. The image contrast on the left showed comparable positive contrast with respect to Gd-DTPA. The slope of the plot 1/T<sub>1</sub> VS Concentration has given the relaxivity r<sub>1</sub> as 7.24 mM<sup>-1</sup>s<sup>-1</sup> for Mn complex and 8.26mM<sup>-1</sup>s<sup>-1</sup> for Gd-DTPA. The r<sub>2</sub> value obtained for the agents are as 41.26 mM<sup>-1</sup>s<sup>-1</sup> for Mn- complex and 11.10 mM<sup>-1</sup>s<sup>-1</sup> for Gd-DTPA. The high r<sub>2</sub> value for Mn-complex is demonstrating towards dual behavior of agent acting positive as well as negative contrast in the two types of imaging pulse sequences. Geissoschizine Methyl Ether in Uncaria Hook (A galenical Constituent of the Traditional Japanese Medicine Yokukansan) has been reported to show permeability towards Blood-Brain Barrier (BBB). Geissoschizine Methyl Ether (GM) has also been shown to have a potent binding to 5-HT<sub>1A</sub> receptors which may be helpful in crossing BBB<sup>3</sup>. In this context, the indole moiety (present as heterocyclic part in GM) in our ligand has been introduced to increase its efficacy in crossing BBB in spite of not following Lipinski's rule. The indole moiety is also found to be involved as a β-amyloid plaque binding moiety in some PET Contrast agents in brain imaging<sup>2</sup>. On the basis of these important factors associated with indole moiety, our reported Manganese contrast agent may be a specific MRI contrast

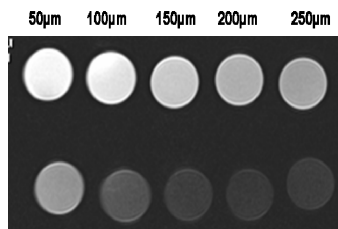
agent for recognition of β-amyloid plaque involved as a hallmark in Alzheimer's disease. We have successfully designed and synthesized a new ligand based MRI contrast agent which is giving a dual contrast in MRI imaging. The dual behaviour in the agent may be due to partial rigid structural entity present in the ligand part providing better water accessibility to the paramagnetic centre Mn<sup>2+</sup>. To the best of our knowledge, it is the first small molecule based MRI contrast agent having dual contrast property.

**Conclusion:** On confirmation of its binding affinity with plaques the synthesized Manganese based MRI contrast agent may be helpful in the diagnosis of Alzheimer's disease in early stages of its progression. Due to its unique property of producing both type of contrast effect, it may be very helpful in obtaining complementary informations in T<sub>1</sub> and T<sub>2</sub> weighted imaging by employing a single agent. Further, work is under progress to modify the above reported MRI contrast agent to be freely water soluble followed by its *in vivo* application in rat models.

**References:** (1) Pan, D., et al. *Tetrahedron* **2011**, *67*, 8431. (2) Qu, W., et al. *F. Bioorg Med Chem Lett* **2008**, *18*, 4823. (3) Imamura, S., et al. *Cell Mol Neurobiol* **2011**, *31*, 787.



T1-Weighted MR images of varying conc. Of (1) Magnitek(Gd-DTPA)(2) Mn-complex



T2-Weighted MR images of varying conc. Of (1) Magnitek(Gd-DTPA)(2) Mn-complex

