

Prague, October 14, 2022

**Review on doctoral thesis „Synthesis of chelators for use in diagnostic imaging“ by Mgr. Jan Kretschmer**

The thesis „Synthesis of chelators for use in diagnostic imaging“ submitted by Mgr. Jan Kretschmer considers highly actual topic of lanthanide chelates for diagnostic imaging with special focus to MRI and PET. The first part of the thesis is focused to  $^{19}\text{F}$  MRI coding with macrocyclic lanthanide chelates containing peptides while the second part is focused to  $^{18}\text{F}$ -radiolabeled gadolinium chelates for PET/MRI. The thesis is logically organized, highly consistent in content and contains lot of very interesting original results. It uses fully appropriate up to date methodology.

Results from the thesis were published in highly prestigious journal Nature Communications, patented and presented on two international scientific conferences. I have only two minor objections to the thesis, which however do not devalue generally excellent level of the thesis:

- 1) Page 37: The compounds are deemed lactate sensors, but obviously also other bidentate hard Lewis base ligands may be coordinated on the sensor. How lactate-selective are the sensors in competition with, e.g., glucuronate, malate, citrate, phosphoesters such as ATP etc. ligands normally present in organism?
- 2) Page 93: Cytotoxicity was measured in the range 1-10 micromolar and was found to be negligible, while the concentrations to be used for imaging on page 94 were 0.05-0.5 millimolar, i.e. much higher (50-500 micromolar). Why cytotoxicity of imaging-relevant concentrations was not determined? In fact, cytotoxicity of both  $[\text{Gd}(\text{FL}^1)]$  and Magnevist should be determined and compared up to concentrations when  $\text{IC}_{50}$  is reached for both compounds to have real comparison of cytotoxicity of these compounds.

I have also few questions:

- 1) For the *in vivo* use, is thermodynamic or kinetic stability of the complexes more important?
- 2) On page 20, there is a mention that free chelator is about as toxic as free metal ion. Which chelator and why?
- 3) The peptides described in the first part of the thesis contain single trifluoromethyl group per relatively large molecule. Which concentration of peptide (in mol/L and mg/L) is already reasonably visible on  $^{19}\text{F}$  MRI phantom? How stable are these peptides towards peptidases omnipresent in the organism?

The thesis has very high quality fully achieving its aims and **I recommend it for defense** as a base of awarding the Ph.D. title to Mgr. Jan Kretschmer with ranking A-excellent.



doc. Mgr. Martin Hrubý, Ph.D., DSc.