**Response to Reviewers JSCS 6866, 2nd**

We have revised our manuscript meticulously following the reviewers’ recommendations. Your comments allowed us to make significant improvements to the manuscript which we believe is now acceptable for publishing. Please see the detailed answer below. Our responses are in blue. In the manuscript the changes are highlighted in red.

**Referee report JSCS 6866, 2nd**

**Title:** Anti-inflammatory activity of synthetic and natural glucoraphanin

**Authors:** Quan V. Vo, Pham C. Nam, Thuc N. Dinh, A. Mechler and Thi T. V. Tran

The manuscript is advanced in comparison to previous and it is less unsaid than previous. But still, there are some issues that should be clarified and explain more precisely. I did not expect that I have to cite every one unsaid thing or every sentence separately, but I expect that authors will understand my objection and correct all insufficient defined part of the manuscript, by themselves.

We have checked the whole manuscript and fixed some errors. We have also rewritten the bioassay part in the comparison of some important published results of anti-inflammatory activity of indole and aromatic glucosinolates. The revised manuscript was also read and corrected by Prof. Mechler (La Trobe University, Australia) who was Dr Vo’s PhD mentor at the time when the data for this manuscript was collected, and who thus joined the authors of the manuscript.

**1.** Abstract, 4th line: here we describe a first total synthesis of *α*-glucoraphanin…

That is not the truth, since authors did not obtain compound **4a**. Please correct the abstract to be in accordance to obtained results and discussion.

The abstract has been rewritten.

**2.** Authors should clarify structure of glucoraphanin, since in the literature it can be find that glucoraphanin is not a salt, but acid, protonated at the N-sulphonate group, with CAS No 21414-41-5. Potassium salt, **4b**, has different CAS No. Authors should clarify did they synthetises glucoraphanin, or corresponding potassium salt. From Experimental part, it could be concluded that they synthetized potassium salt. That is OK, but corresponding correction in the text should be done. In contrast, protonation step have to be included in Experimental part, and all corresponding corrections that proceed from that. Furthermore, biological test done with β-glucoraphanin or with corresponding potassium salt?

Glucosinolates are known as anionic species (*Phytochemistry* ***56*** *(2001) 5-51*), as the sulfonic acids are strong acids and hence mostly dissociated in aqueous environments. Thus, the purified solid is typically a salt, unless the compound is purified from a highly acidic environment. Therefore, in un-buffered aqueous solution the difference between the acid and the potassium salt is nil.

When considering the purification, it was found that potassium salts are more stable; therefore most of synthetic glucosinolates were purified as potassium salts (e.g. *C. R. Chimie 14 (2011) 194–210*). Consistently the beta-glucoraphanin was synthesized as potassium salt, following our success in the total synthesis of other glucosinolates (*Car. Res.*, 2018, 455, 45-53; *Tetrahedron*, 2013, 69, 8731-8737; *Bioorg. Med. Chem.*, 2013, 21, *5945-5954; Bioorg. Med. Chem.*, 2014, 22, 856-864).

Bioassays are normally conducted under buffered conditions at pH 7.4 (*Planta (2017) 246:19–32; Anal. Methods, 2010, 2, 310–325*) thus, in the testing conditions, glucosinolates exist in anionic form which determinate bioactivity of glucosinolates. Consistently in our study the anti-inflammatory activity was tested with the β-glucoraphanin potassium salt at pH ≈ 7.4, where glucoraphanin is anionic. Thus from the point of the bioassay, it is irrelevant if the acid or the salt was used. In regards to the synthesis, the manuscript has been corrected describing glucoraphanin potassium salt as the final product.

**3.** Scheme 1 should be corrected:

a) Since *α*-glucoraphanin was not obtained, only structure for *β*-glucoraphanin (**4b**) should remain.

The scheme 1 has been redrawn, the compound **4a** has been omitted.

b) Quotation of compounds should be given in more clarified manner, for purpose to avoid some misunderstanig which I pointed out in previous report. My suggestion is that authors, instead to said „α/β”, or „2a,b” (3a,b) write „α or β”, „2a or b” and changes it in that manner Scheme 1 and the whole text.

The paragraph has been rewritten, all of the values “α/β” or “a,b” have been separated as the advice. The **4a** compound was replaced by α-GRP potassium salt to avoid misunderstanding in the manuscript.

Minor corrections are necessary:

This manuscript should be accepted for publication as Communication after minor corrections without my additional reading.