XANTHONOLIGNOIDS INHIBITORS OF PROTEIN KINASE C – SEMI-PREPARATIVE ENANTIORESOLUTION ON POLYSACCHARIDE CHIRAL STATIONARY PHASES WITH A SOLID-PHASE INJECTION SYSTEM

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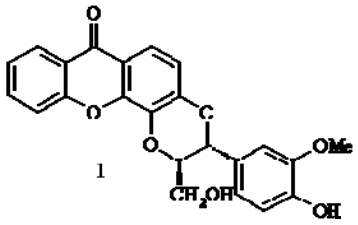
Kielcorins are 1,4-benzodioxane derivatives belonging to the xanthonolignoids [1]. We have recently described the synthesis [2] and the analytical enantiomeric resolution by chiral HPLC [3] of four racemic xanthonolignoids: *rac-trans*-kielcorin C (1), *rac-trans*-kielcorin D (2), *rac-trans*-isokielcorin D (3), and *rac-trans*-kielcorin E (4). As the racemates of kielcorins 1-4 demonstrated an antiproliferative effect [2] and also an effect compatible with PKC inhibition [4], the resolution of the racemates at a preparative scale seemed desirable.



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In order to evaluate the effect of the separated enantiomers of xanthonolignoids on isoforms α, βI, δ, η and ζ of protein kinase C (PKC), milligram amounts were obtained by semipreparative HPLC employing a *tris*-3,5-dimethylphenylcarbamate amylose phase using polar organic conditions [3] and multiple injections. The poor enantioselectivity factor (α= 1.3) obtained for *rac-trans*-kielcorin C (1), associated to its low solubility even in polar organic conditions, led to a labor-intensive multimilligram separation. Thus, this communication presents an innovative system to increase the load amount of the sample by a solid-phase injection, instead of the

classical volume injection into the loop. The injection system consisted of a pre-column in which the solid substance is mixed with silica. The closed-loop recycling chromatography was associated with this new technique in order to maximize the throughput and productivity for *rac-trans*-kielcorin C (1).

Solid-phase injection increased the productivity and yield of the preparative process, being a valuable alternative in preparative HPLC for low-solubility samples when compared to classical sample injection.

The effect of the enantiomers on isoforms α, βI, δ, η and ζ of PKC was studied using an *in vivo* yeast assay and their effects compared with those obtained with the racemates 1-4. In general, these compounds behaved as potent PKC inhibitors of the novel PKC isoforms (δ and η) and the atypical PKC-ζ. All the enantiomers investigated were inactive on the classical isoforms (α and βI). Differences on the potency and enantioselectivity towards an individual PKC isoform were noted when comparing the activities between the enantiomeric pairs. For instance, (+)-*trans*-kielcorin C (1) was a potent and selective PKC-η inhibitor while its antipode (+)-*trans*-kielcorin C (1) showed to be inactive.

[1] Pinto, M. M. M.; Sousa, E. P. *Curr. Med. Chem.* **2003**, *10*, 1-12. Pinto, M. M. M.; Sousa M. E.; Nascimento, M. S. J. *Curr. Med. Chem.* **2005**, *12*, 2517-2538.

[2] Sousa, E. P.; Silva, A. M. S.; Pinto, M. M. M.; Pedro, M. M.; Cerqueira, F. A. M.; Nascimento, M. S. J. *Helv. Chim. Acta* **2002**, *85*, 2862-2876.

[3] Sousa, E. P.; Tiritan, M. E.; Oliveira R. V.; Afonso, C. M. M.; Cass, Q. B.; Pinto, M. M. M. *Chirality* **2004**, *16*, 279-285.

[4] Saraiva, L.; Fresco, P.; Pinto, E.; Sousa, E.; Pinto, M.; Gonçalves, J. *J. Enzyme Inhib. Med. Chem.* **2003**, *18(4)*, 357-370.

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