SYNTHESIS, RADIOLABELLING AND EVALUATION OF N^5-[^{18}F]FLUOROETHYL-PIRENZEPINE AND ITS METABOLITE N^5-[^{18}F]FLUOROETHYL-RS 75

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Introduction and aim: Pirenzepine 3, namely 11-[2-(4-methyl-piperazin-1-yl)-acetyl]-5,11-dihydro-benzo[e]pyrido[3,2-b][1,4]diazepin-6-one has originally been developed as M_1 selective muscarinic antagonist. Recently it has been shown[3] that 3 or its metabolite exhibit an unusual behaviour *in vivo*, that cannot be explained with M_1 antagonism. 3 undergoes metabolism *in vivo* to form LS-75 4 5,11-dihydro-benzo[e]pyrido[3,2-b][1,4]diazepin-6-one. Compound 4 was found to be a moderate inhibitor of PARP, an enzyme directly related to e.g. neuronal signal transduction and in particular to the regulation of key events in apoptotic cascades[3] Lesioned brain regions show an elevated concentration/activity of PARP-1, indicating links to neurodegenerative disorders. We were interested to investigate this second pirenzepine-related mode of action on a physiological level[2]. Our aim was to synthesise appropriate ^{18}F-fluorinated analogues of both compounds in order to provide the tools for an *in vivo* PET-study in healthy Sprague-Dawley rats of these potentially beneficial side effects of pirenzepine, which are beyond pure M_1 antagonism. In addition, the muscarinic acetylcholine receptor have been studied extensively with regard to its relation to several disorders including Alzheimers disease.

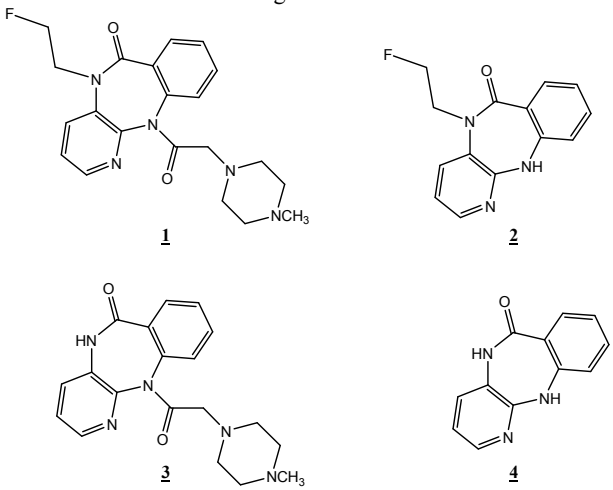
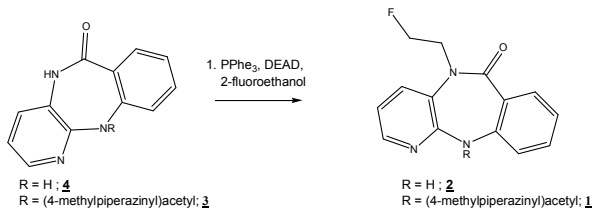


Fig. 1: parent compounds and fluoroalkylated congeners



Methods: 4 was prepared from ethyl-2-amino-benzoate and 3-amino-2-chloro-pyridine. 3 was obtained by reacting 4 with 2-chloro acetic acid chloride followed by 4-methyl piperazine. Exposure to sodium ethoxide in ethanol yields sodium amides **3a** and **4a**. Alkylation of 3 and 4 with 2-fluoroethanol under Mitsunobu conditions afforded reference compounds 1 and 2 in yields of 95 and 90 % respectively. **3a** and **4a** have been labeled employing 2- [^{18}F]fluoroethyl tosylate. [^{18}F]-1 and [^{18}F]-2 were isolated by solid phase extraction, purified by HPLC and formulated in PBS prior to application. [^{18}F]-1 was isolated in a specific radioactivity of 12,5 MBq / nmol. For autoradiography, brain sections from adult, male Sprague-Dawley rats were used. Prior to incubation with radioligands, sections were pre-incubated in assay buffer (50 mM Tris/HCl buffer, pH 7.5, containing 120 mM NaCl). Nonspecific binding was determined using 100 µM of pirenzepine. Assay: Addition of 125 µl [^{18}F] 10 MBq/ml, 80 phosphate buffered saline.Incubation 60 min in trisHCl-buffer (120 mmol NaCl) at pH 7.5, washed two times for 10 s, detection on storagephosphor over night.

Results and Discussion: Both pirenzepine and its metabolite RS 75 have been prepared and labeled with ^{18}F via 2-[^{18}F]fluoroalkylation in position 5 of the benzodiazepinone moiety to obtain N^5-[^{18}F]fluoroethyl pirenzepine and N^5-[^{18}F]fluoroethyl LS 75. After autoradiographic evaluation, [^{18}F]-2 can now be evaluated in animal studies in vivo using Sprague-Dawley rats and a small animal PET scanner. [^{18}F]-2 may be considered as a promising candidate for quantitative visualisation of PARP-1 distribution in rodent model.

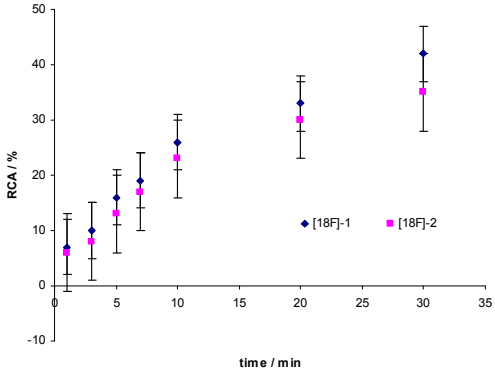


Fig. 2: Radiochemical yield as a function of time. Conditions: DMSO, K_2CO_3, 120 °C

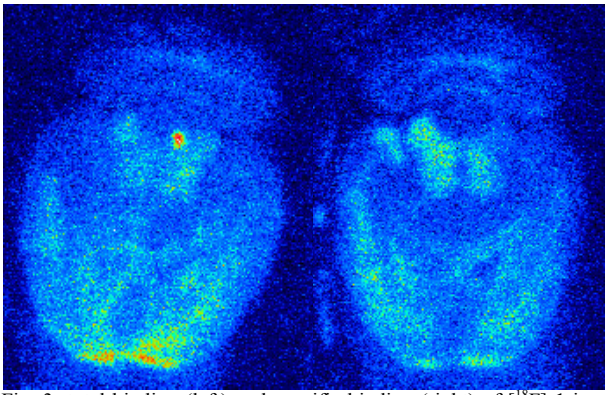


Fig. 3: total binding (left) and specific binding (right) of [^{18}F]-1 in a coronal section of adult sprague-dawley rats

References

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[2] A Schrattenholz, V Soskic; Current Topics in Medicinal Chemistry 6 (2006), 663-686
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