Design, Synthesis and Characterization of a Selective Tritium-labeled $P2Y_{12}$ Receptor Antagonist

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The $P2Y_{12}$ receptor expressed on platelets is a target of antithrombotic drugs (e.g. clopidogrel and ticagrelor).[1] In addition, it is expressed in the brain on microglial cells and involved in neuroinflammation.[2] Radioligands, e.g. positron emission tomography (PET) tracers, targeting $P2Y_{12}$ receptors have potential for diagnostic imaging and therapeutic monitoring providing a non-invasive method to study receptor expression and distribution as well as monitoring activated microglial cells in vivo.[3-4]

In this study, we set out to develop a non-nucleotidic, potent and selective radioligand for the labeling of $P2Y_{12}$ receptors, with potential to penetrate into the brain. A comprehensive data analysis of published $P2Y_{12}$ receptor antagonists was conducted and key parameters such as binding affinity, selectivity, pharmacokinetic properties, and properties related to brain bioavailability were evaluated. This led to the selection of a promising $P2Y_{12}$ receptor antagonist scaffold,[5] and the design and synthesis of a tritium-labeled $P2Y_{12}$ receptor antagonist, termed [^{3}H]PSB-22219. The new radioligand displayed high-affinity binding to membrane preparations recombinantly expressing the human $P2Y_{12}$ receptor (K_D = **4.57** nM) and low non-specific binding (< 10 % of total binding), while non-transfected cells were devoid of specific binding sites for the radioligand. Selectivity of [^{3}H]PSB-22219 was confirmed versus the closely related receptor subtypes $P2Y_1$ and $P2Y_{13}$. The established radioligand binding assay was employed to characterize $P2Y_{12}$ receptors natively expressed in human platelets (K_D = **2.53** nM) and rat brain cortical membrane preparations (K_D = **5.35** nM). This new, superior radioligand is expected to become a useful pharmacological tool. It will contribute to the future development of PET ligands and therapeutics targeting brai $P2Y_{12}$ receptors.

(1) Y. Baqi, C. E. Müller, *Drug Discov. Today,* **2019**, *24*, 325–333.

(2) A. Mansour, C. Bachelot-Loza, N. Nesseler, P. Gaussem, I. Gouin-Thibault, *Int. J. Mol. Sci.,* **2020**, *21*, 1391.

(3) A. Mildner, H. Huang, J. Radke, W. Stenzel, J. Priller, *Glia,* **2017**, *65*, 375–387.

(4) C. E. G. Uff, K. Patel, C. Yeung, P. K. Yip, *Biomolecules,* **2022**, *12*, 603.

(5) C. Boldron, A. Besse, M. F. Bordes, S. Tissandié, X. Yvon, B. Gau, A. Badorc, T. Rousseaux, G. Barré, J. Meneyrol, G. Zech, M. Nazare, V. Fossey, A. M. Pflieger, S. Bonnet-Lignon, L. Millet, C. Briot, F. Dol, J. P. Hérault, P. Savi, G. Lassalle, N. Delesque, J. M. Herbert, F. Bono, *J. Med. Chem.,* **2014**, *57*, 7293–7316.