Avis favorable

Dr. Cyril Ollivier

Dr Cyril OLLIVIER
Directeur de l'Ecole Doctorale
de Chimie Moléculaire

Campagne 2020 Contrats Doctoraux Instituts/Initiatives

Proposition de Projet de Recherche Doctoral (PRD)

Appel à projet ISim - Initiative Sces et ingénierie moléculaires 2020

Intitulé du Projet de Recherche Doctoral : Design and pharmacological evaluation of neuropsin inhibitors as therapeutics and potential diagnosis tools for Alzheimer's Disease and associated dementias

Directeur de Thèse porteur du projet (titulaire d'une HDR) :

NOM : **BOTUHA** Prénom : **Candice**
Titre : Maître de Conférences des Université
ou
e-mail : candice.botuha@sorbonne-universite.fr
Adresse professionnelle : Sorbonne université, Campus Pierre et Marie Curie, 4 place
(site, adresse, bât., bureau) jussieu 75005 Paris case 229, tour 33-43 bureau 404

Unité de Recherche :
Intitulé : Institut Parisien de chimie moléculaire, IPCM
Code *(ex. UMR xxxx)* : 8232

ED406-Chimie Moléculaire Paris Centre

Ecole Doctorale de rattachement de l'équipe & d'inscription du doctorant :

Doctorants actuellement encadrés par le directeur de thèse (préciser le nombre de doctorants, leur année de 1ere inscription et la quotité d'encadrement) : 2 thèses en cours, 1- Doctorante MENRT, 1ère inscription 2018 , encadrement 33%; 2-doctorant IPV, 1ère inscription 2018, encadremen 33%. d

Co-encadrant :

NOM : **El Amri** Prénom : **Chahrazade**
Titre : Professeur des Universités ou HDR ☒
e-mail : chahrazade.el_amri@upmc.fr

Unité de Recherche :
Intitulé : IBPS, Adaptation Biologique et Viellissement
Code *(ex. UMR xxxx)* : 8256

ED515-Complexité du Vivant

Ecole Doctorale de rattachement : Ou si ED non Alliance SU :

Doctorants actuellement encadrés par le co-directeur de thèse (préciser le nombre de doctorants, leur année de 1ere inscription et la quotité d'encadrement) : 2 thèses en cours, 1- 1e inscription octobre 2017 (100%), soutenance prévue automne 2020; 2- doctorante IPV (encadrement 50%) 1e inscription 2018.

Cotutelle internationale : ☒ Non ☐ Oui, précisez Pays et Université :

Description du projet de recherche doctoral *(en français ou en anglais)*

3 pages maximum – interligne simple – Ce texte sera diffusé en ligne

General context of the project

In France about 1.9 million people suffer from Alzheimer's type dementia, and 35 million patients worldwide. The challenges of Alzheimer's disease (AD) research are to diagnose the disease before the onset of irreversible brain damage services. Very few robust molecular biomarkers are available to date, we propose in this project to focus on an emerging serine protease biomarker. The involvement of serine proteases in the CNS physiology and neurodegenerative diseases like AD is continuously highlighted.[1] Among them, kallikrein related-peptidases (KLKs) are coded by 15 structurally similar genes which co-locate in tandem on chromosome 19q13.4, and represent the largest cluster of contiguous protease genes in the human genome. In the framework of the PhD project, we will focus on **KLK8** (also called neuropsin) a tryptic serine protease. **KLK8** is expressed in the hippocampus, the lateral nucleus of the amygdala as well as other areas of the limbic system that are all involved in learning and memory.[1] The activity of stress-induced **KLK8** leads to increased cleavage of the EPHB2 receptor (Ephrin type B receptor 2) on the surface of amygdala neurons, and promotes anxious behaviors in mice. In a murine model of AD, the inhibition of **KLK8** restored normal cognitive functions while activating pathways of clearance of the Aβ peptide.[2] Tight regulation is thus warranted to prevent unopposed proteolytic activity that may lead to pathological processes. **Recently, it has been suggested that both cerebrospinal fluid (CSF) and blood KLK8 may serve as a novel biomarker for diagnosis of AD at incipient stages**.[3] Moreover, in a reverse *in vitro* approach, **KLK8** induction reduced EPHB2 and total tau and increased the ratio of phospho-tau/total tau, leading to impaired proliferation and neuronal differentiation.[4] These findings underline the therapeutic potential of **KLK8** inhibition by counteracting plasticity deficits in AD-affected brain.

To date, despite the growing interest for this target, none inhibitors have been reported in contrast to other kallikrein-related peptidases.[5] Recent results obtained by the co-supervisors in collaboration allowed to select two hits compounds with low-micromolar IC_{50} with distinct mechanisms of action *eg* reversible and covalent inhibitions. These two hits are structurally different: the first is deferasirox (**DFX**), an iron chelator used in clinic as Exjade®, which possesses two phenolic units attached to a 1,2,4-triazoles core.[7] The second compound belongs to the family of 4H-pyrido[e][1,3]oxazin-4-ones (**PyrOx**), a new class of heteroaromatic rings which has been synthetized in three steps and poly-functionalized by the supervisor's team (Chembio team).[8] Interestingly, the high reactivity of **PyrOx** toward nucleophiles has allowed the supervisor chemists to use **PyrOx** as molecular scaffold to synthetize other small heterocycles of interest such as 2-hydroxypyridyl 1,2,4 oxadiazoles, 1,3,5 triazines and 1,2,4 triazoles (**PyOHTr**), the latter corresponding to aza analogs of **DFX**.[9] Thus, a chemical library of molecules has been established using a well-managed synthetic strategy, all compounds possessing either a pyridoxazinone or a 1,2,4 triazole as a central core with high chemical diversity. However, structural modification could be envisioned with low risky level in order to optimize their inhibition profile *via* a structure activity-relationship (SAR) studies (see methodology).

This project is ideally included in the ISim call for projects since we propose an elaborated molecular approach gathering two research teams with complementary skills to design and select lead compounds as therapeutics and/or activity-based probes for early diagnosis of AD by taking advantage of KLK8 emerging biomarker. Namely, this interdisciplinary project at the interface of medicinal chemistry and biology with a high potential in translational research will be based on *in vitro* evaluation using medicinal chemistry, protease enzymology concepts, preclinical cell and mouse models of AD.

Main outlines of the PhD project (Scheme 1)

C. Botuha (supervisor 1, PI) is an expert in organic synthesis and synthesis of heteroaromatic compounds. She has also a good expertise in medicinal chemistry. She has experience in management of 4 PhD (2 MENRT, 2 IPV projects, 1 postdoc (LabEx MIChem 2018). The supervisor team (Chembio) has already set up a collaborative work with biologists co-supervisor team (B2A) (IPV

2015-2018).[9c] This fruitful collaboration allowed both teams to already develop a strong partnership gathering their complementary know-hows.

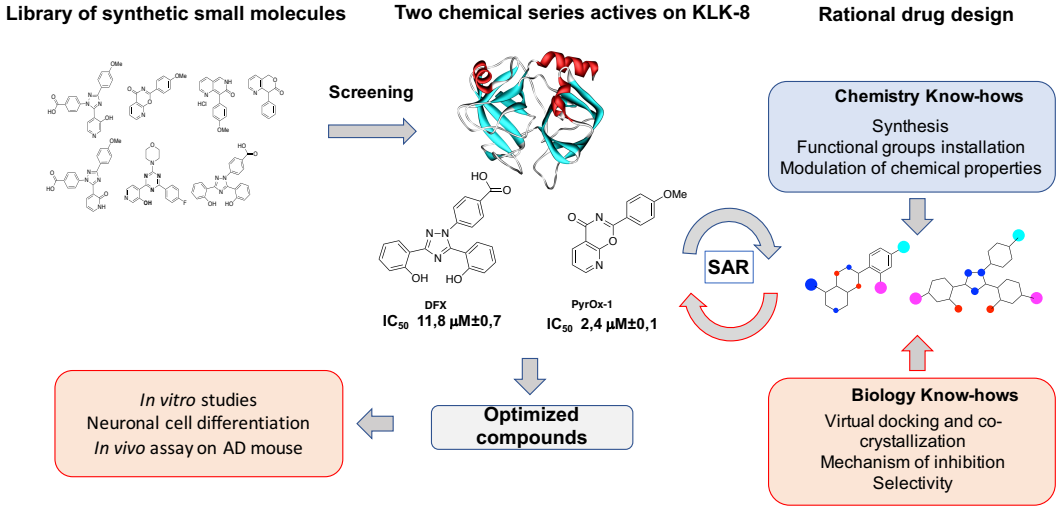
C.El Amri (Co-supervisor 2) has a strong expertise in enzymology of serine proteases, design and mechanistic characterization of inhibitors targeting KLKs, with 4 PhD supervised in this theme, 4 patents since 2012 (Soualmia *et al.*, 2018[10]; Masurier *et al.*, 2018[5], El Amri *et al.*, Patent 2018, 2020[11]).

1. Design, chemical synthesis and optimization of inhibitors. C. Botuha (supervisor 1, PI)

Following a conventional drug discovery process, we will measure the influence of structural modifications of **PyrOx** compounds on inhibition activities (SAR). Focus will be on synthetizing heterocycles containing substituent with different electronic and steric nature. Thus, we will design and synthetize a small library of diversely substituted **PyrOx** using the well-managed 2 steps synthetic strategy developed by chemist's supervisor team.[9] The modulation of the chemical properties of **PyrOx** will be also achieved via post-functionalization of the halogen-substituted heterobicycle using Pd(II) catalyzed cross-coupling reactions.[9c] isomers of **PyrOx** with different positions of the nitrogen atom on the pyridine ring will be investigated in order to evaluate the impact of structural change of the central ring on polarity, lipophilicity and hydrophilicity. If necessary, other original heterobicycles based on oxazinone scaffold (pyrimido- and pyrazinoxazinone …) might be considered in this purpose. A recent structural biology study provides a comprehensive picture of the molecular mechanisms underlying the enzyme activity of **KLK8** which constitutes a very useful basis for the design of KLK8 modulators.[6] We will thus next perform an exhaustive screening of functionalized compounds to select hit compounds with optimized inhibition activities. In house virtual docking and co-crystallisation in collaboration with Peter Goettig (Department of Molecular Biology, University of Salzburg) of selected hit compounds with KLK8 will be performed to help for the optimization. In the meantime, design and synthesis of analogs of **DFX**, based on 2-hydroxy-benzo(pyrido)-1,2,4 triazoles will be performed starting from the corresponding benzo(pyrido)oxazinones. Using the same drug design methodology as **PyrOx**, we will select hit compounds based on **DFX** with optimized inhibition activities.

2. Biological evaluation of KLK8-targeted inhibitors: selection of hit compounds and preclinical study. C.El Amri (Co-supervisor 2)

In vitro* evaluation by enzymatic kinetic analyses** (inhibition quantitation (IC_{50}, K_i, k_{inact}/K_I for covalent inhibitors) and mechanistic characterizations) using fluorescence assays with peptide fluorogenic model substrate (VPR-AMC) and substrates reproducing the main biological substrates (PAR and EPHB2 receptor's peptide derivatives). The *in vitro* mechanistic studies will be performed with compounds displaying appropriate inhibitory potency typically IC_{50} below 10µM. First the reversible or irreversible nature of the inhibitions will be examined using dilution method for KLK8. We will then check for cross-inhibition within a large set of serine proteases both challenging in the CNS (KLK6, tPA, thrombin, trypsin, mesotrypsin, KLK11) but also other kallikreins (KLK3, KLK4, KLK5, KLK14), inflammatory proteases involved in CNS inflammation (Caspase 1; Cathepsin C). Molecular docking studies on KLK8 will be also performed to establish structural basis of the inhibition. **Preclinical study*: The effect of the selected lead compounds will be then evaluated for their ability to constitute good disease-modifiers using AD's *in vitro* and *in vivo* models. 1- Cytotoxicity towards various neuronal cells (hippocampal, cortex, striatum) and glial cells (microglia, oligodendrocytes, astrocytes) will be checked prior to further evaluation. 2- The selected leads will be then assayed against hippocampal neuronal cells to check for their capacity to promote their differentiation especially based on the activation of EPHB2 receptor by KLK8 (neuronal cell lines and hippocampal primary neurons). *In vivo* evaluation on AD mouse model (B2A) will be performed in the final step for inhibitors with the best pharmacological profiles (selectivity, no cytotoxicity, pro-differentiation potential).



Scheme 1

Expecting results and positioning

The diagnosis of AD is presently going through a paradigm shift toward the implementation of biomarkers to support identification of predementia and even preclinical asymptomatic stages of the disease. This represents an important public health issue. Particularly, accuracy in this early diagnosis may be improved by use of cerebrospinal fluid biomarkers and innovative imaging. With this project, we thus propose an elaborated molecular approach to design, evaluate and select lead compounds as therapeutics and/or activity-based probes toward Neuropsin an emerging biomarker for early diagnosis in AD. Resulting compounds with optimized pharmacological profiles will be patented.

Student profile

The PhD fellow will be involved both in the design/synthesis and the biological evaluations of the new KLK8 inhibitors. The research will be conducted simultaneously in both teams at IPCM and at B2A (Biological Adaptation and Aging research Unit). We are looking for a strongly motivated student holding a master M2 in molecular chemistry with at least experience in synthetic organic chemistry and medicinal chemistry researches. An experience in Biology would be appreciated through the validation of Biology courses (Biochemistry, cell biology…).

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