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Synthesis of novel alpha7-nAchR ligands : from an idea to in rodent results for Alzheimer [^{18}F] TEP imaging

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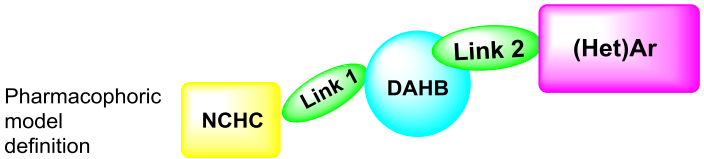
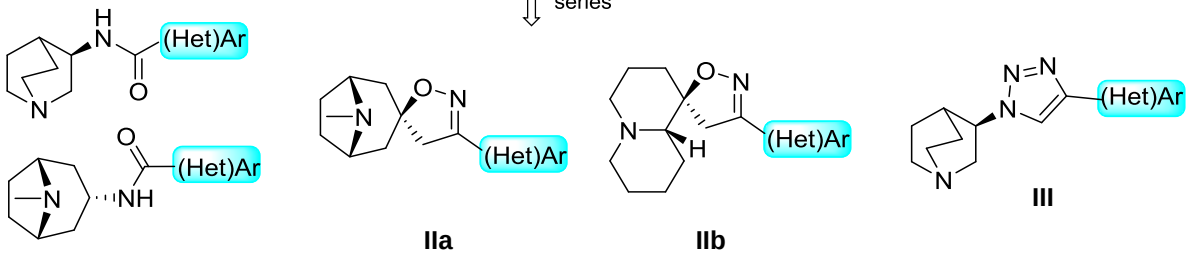
Synthesis of novel alpha7-nAchR ligands : from an idea to in rodent results for Alzheimer [^{18}F] TEP imaging



Designed and synthesized series



I IIa IIb III

(Het)Ar : phenyl, thiophene, pyridine, benzofurane, benzothiophene, indole...

- α7 nAchR Ki detemination
- SAR analysis
- QSAR
- radiolebeling in vivo studiez

- 57 ligands with 10 < Ki < 50 nM
- 37 ligands with Ki < 10 nM
- 12 lignads with Ki < 1 nM
- Total Selectivity toward $\alpha 4\beta 2$
- $5HT_3$ binding in the nanomolar range

Abstract:
The neurotransmitter acetylcholine (ACh) exerts its effects on the central nervous system (CNS) through two distinct muscarinic mAChRs and nicotinic nAChRs receptors types: nAChRs belong to the superfamily of ligand-gated ion channels possessing a pentameric structure. Because of their distribution and abundance in the CNS (in particular in the hippocampus and cortex), the a7 subtypes are potential diagnosis and therapeutic targets for brain disorders that involve these cerebral regions. Having in hand a human compatible [^{18}F]-labeled positron emission tomography (PET) tracer to realize the early diagnostic or to validate the efficiency of therapies in clinical trials for AD is indubitably crucial.

In this aim, based on our expertise in heterocyclic bio-mimetic development, we also envisioned to design novel α7 nAChR ligands and their transformation into a [^{18}F] PET tracer. We synthesized a library of potent α7 nAChR ligands containing a quinuclidine, a tropane or a 8*H*-quinolizine moiety. We present herein chemistry, SAR studies, *in vitro* efficiency (SAR), radiolabeling and i*n vivo* results in rats.

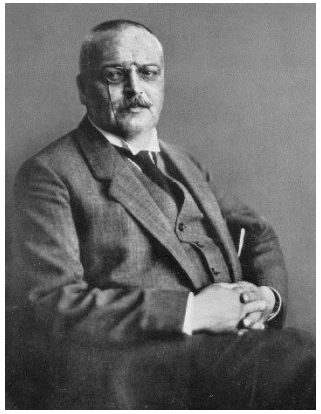
Keywords: quinuclidine, tropane, amide, triazole, alpha 7 nAchR ligands, Synthesis, SAR, radiolabelling, in vivo results

Introduction

In brief : Dementia

First observations in brain :

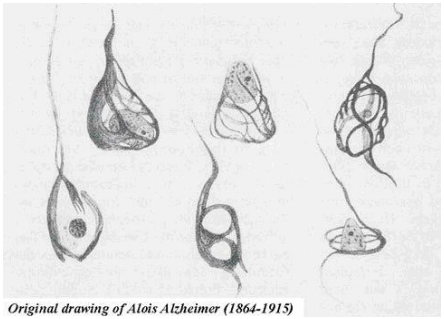
- Extra neuronal dense deposits (Amyloid plates).
- Characteristic intracellular inclusion, synomynous of a **neurofibrillary *degeneration***.



Aloïs Alzheimer
(1864-1915)

Qualification of dementia:

- **Rare** for young patient
- **Senile dementia** for old persons



Original drawing of Alois Alzheimer (1864-1915)

First schematic representation of brain elements in AD brain

Introduction

Consequences are important:

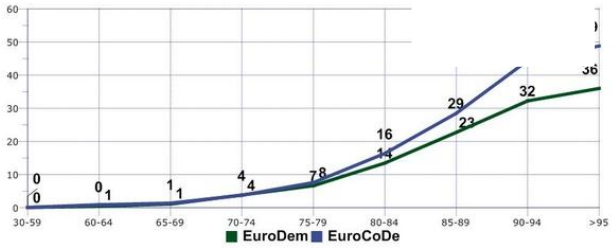
- Degeneration of brain tissue
- Progressive and irreversible loss of mental functions
- Progressive loss of memory, langage, cognition and mouvement
- Accompanying with a cerebral nervous cell destruction

An incredible incidence :

- 36 millions worldwide persons affected by dementia in 2010 and…..
- Probably 66 millions in 2030, 115 millions in forthy years (2050)…..

- 1 % between 65 and 69 years old,
- 20 % between 85 and 89 years old ,
- 40 % more than 90 years old in Europe .

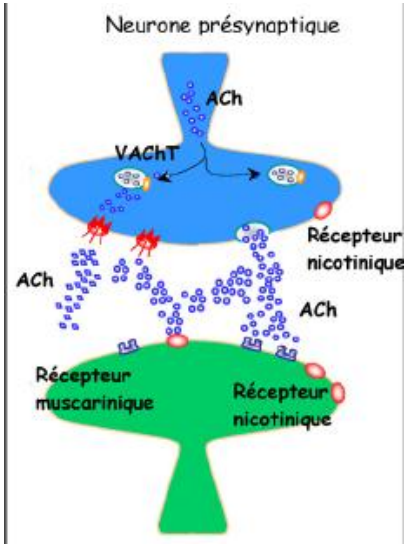
Prevalence of dementia (women)



Introduction

Example of biological targets identified since the discovery of the desease

Synapse



Muscarinic Receptors

Transport TSPO

NMDA receptors

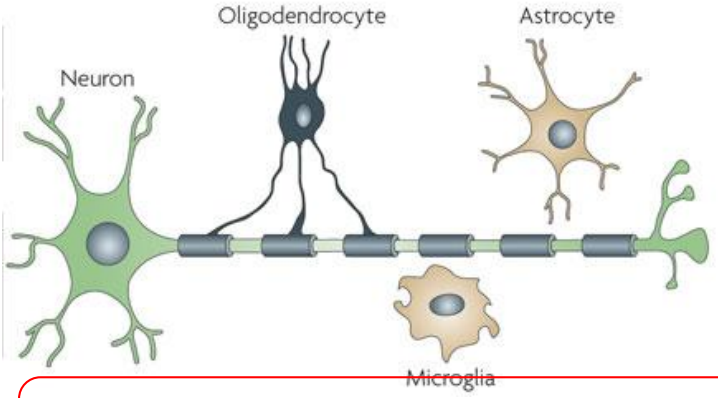
Nicotinic Réceptors

Ach Esterase

Follow the neuronal activity ?

Solution : PET imaging

System



Inflammation
Oxygenated reactive species ROS

Neurofibrillary degeneration :
Tau protein tangles
β Amyloid deposits

Aβ and Tau associate enzymes
BACE (α secretases)
Kinases (CDK5, GSK3, CK1, DYRK1A…)

Introduction

A diagnostic approach For AD:

^{18}F PET imaging (positron emission tromography)

- For an efficient design of PET ligands :

1) the development of drug interacting with a biological target is required with
- Structure / affinity relationship etablishment (SA_fR)
- Low toxicities of final molecules.

2) The introduction of radio nucleide is performed with compatible clinical methods
- Mainly under S_N ou S_NAr reactions

3) The introduction of radio element will be achieved without any affinity decrease (require novel SA_fR)

4) Probes will answer to stablility, efficiency and reproductibility criteria

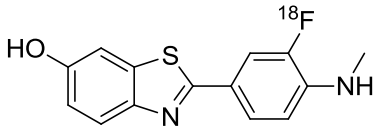
Introduction

Ideal characteristics of a tracer:

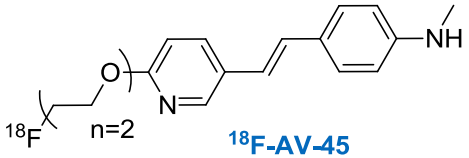
- High affinity and selectivity for the biological target
- Moderated lipophilicity for a brain penetration (BBB).
- Low molecular weight (< 500 DA).
- High in vivo stability (reduction of radioactive metabolites production)

Some examples:

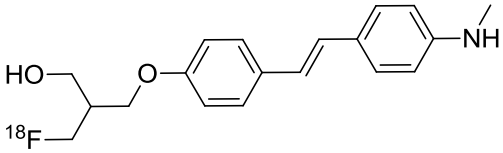
β A



^{18}F-Flutemamol

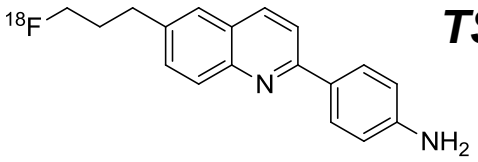


^{18}F-AV-45
Florbetapir



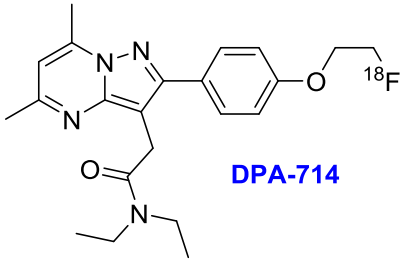
^{18}F-FMAPO

Tau



18F-THK523

TSPO

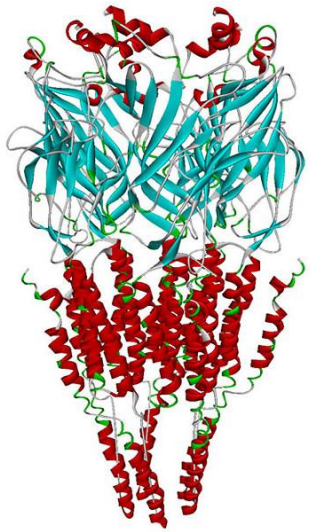


DPA-714

AD pathology
In progress

Current Medicinal Chemistry, **2007,** *14*, *Seminars in Nuclear Medicine*, **2012**, *42*, 423-432.

Introduction



Our objective : target the alpha 7 n Ach R receptor and design 18F imaging probes

Recent work has demonstrated a potential role in reducing inflammatory **neurotoxicity** in stroke, myocardial infarction, sepsis, and **alzheimers disease**.

α7 **nicotinic receptors** appear to be critical for **memory**, **working memory**, **learning**, and **attention**

α_7 nicotinic agonist appears to have positive effects on neurocognition in persons

Brain distribution :

- hippocampus,
- cortex,
- cerebellum,
- olfactory bulb,
- striatum,
- thalamus,
- spinal cord

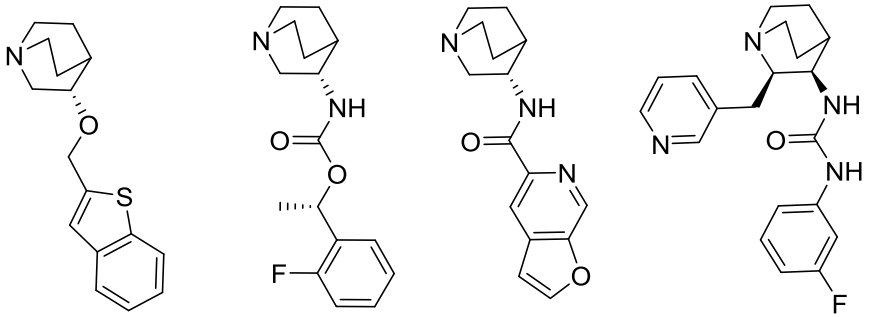
Having an alpha 7 ^{18}F tracer will:

a) Help to identify their exact role in AD progression
b) Offer a tool for early diagnostic of persons
c) Be a solution to validate and quantify benefits to patients during prophilactic and therapeutic strategies.

Introduction

Models :

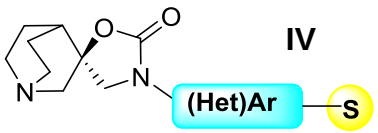
Quinuclidine/amide like

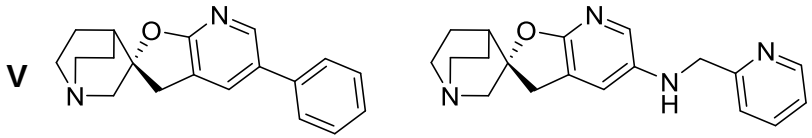


Ether | **Carbamate** (JN403) | **Amide** (PHA-543613) | **Urea**

Bioorg. Med. Chem. Lett., **2004**, *14*, 3781, *J. Med. Chem.*, **2005**, *48*, 905, B*ioorg.Med. Chem.*, **2006**, *14*, 8219., *Biochem. Pharmacol.*, **2009**, *78*, 803.

Spiranic derivatives





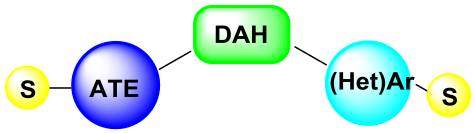
(Het)aryles residues tolerated
Best results with thiophenes Sat = Cl, Br, CN
indoles avec Sat = alkyles
Ki between 2-50 nM

Tricyclic strutures are less described
Phenyles or amino alkyles tolerated
Ki between 3-10 nM

J. Med. Chem. **2005**, *48*, 2678 ; *J. Med. Chem*. **2008**, *51*, 6293 ; *Bioorg. Med. Chem. Lett*. **2005**, *15*, 4727.

Objectives

Pharmacophoric model :



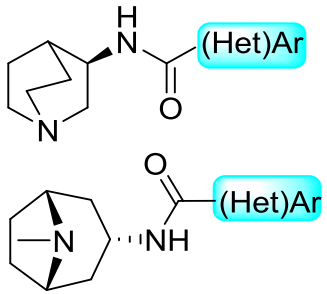
ATE : A tertiary amine .

DAH : an donnor / acceptor of hydrogen bond group .

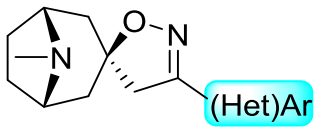
(Het) Ar : an (het)aromatic fraction

S : some satellites could be added to modulate affinity and to incorporate the fluorine atom

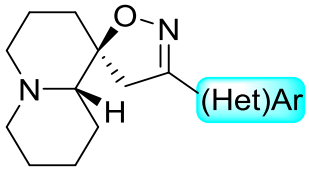
Families I-III are chosen to understand SAR and to create novel ligands:



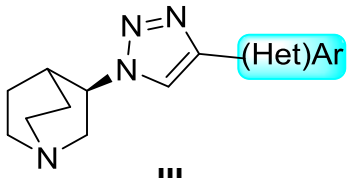
I



IIa



IIb

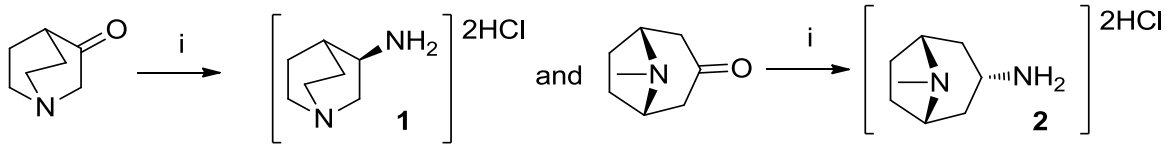


III

Results and discussion

Amid series I : SAR understanding and preparation of references

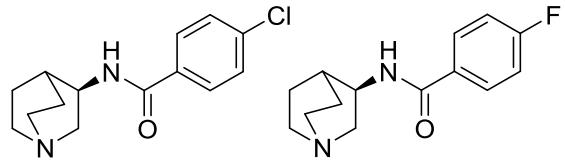
1) Primary amine synthesis



i) HCO_2NH_4 (10.0 eq.), Pd(C) 10% cat., MeOH/water 9/1, r.t., 12 h then aq. 37% HCl, EtOH, 0°C, 2 h, for **1** quant., for **2** 98 %.

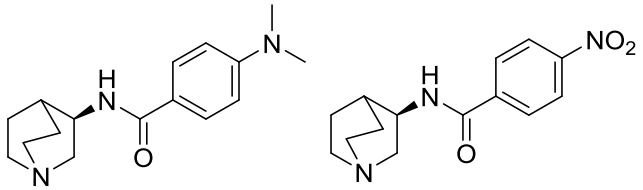
2) Amidification

From amines **1** ou **2**,
Carboxylic acid (1.5 eq.),
DCC (1.5 eq.),
DMAP (cat.),
Et_3N (3.0 eq.),
CH_2Cl_2, rflx, 6h.



3 (74%),
Ki = 17 nM

4 (68%),
Ki = 18 nM



5 (60%),
Ki = 158 nM

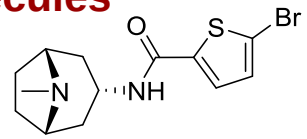
6 (74%),
Ki = 44 nM

- **10 molecules**
- **In quinuclidine series Ki values similar to litterature : Tests validated**
- **Noteworthy : 6 will afford the ^{18}F 4 by S_NAr**

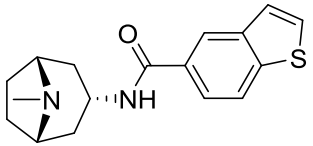
Results and discussion

Amid series I :
Tropanes Aryl amides (novelty)

- **Less active molecules**



7 (80%),
Ki = 78 nM



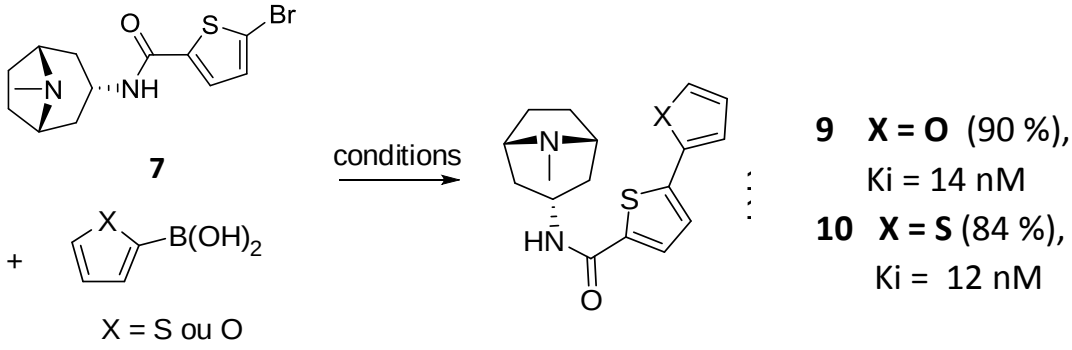
8 (77%),
Ki = 108 nM

- **But**

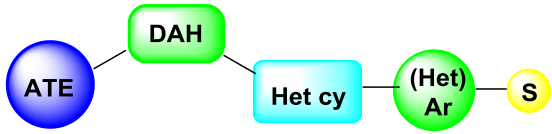
A supplementary palladium catalyzed arylation restored the affinity

Experimental conditions .

Boronic acid (1.2 eq.),
$Pd(PPh_3)_4$ (10%),
K_2CO_3 (2.0 eq.),
Toluene/EtOH (3/1),
microwave irradiation,
150°C,
20 min.



- **The pharmacophoric model could be refined**



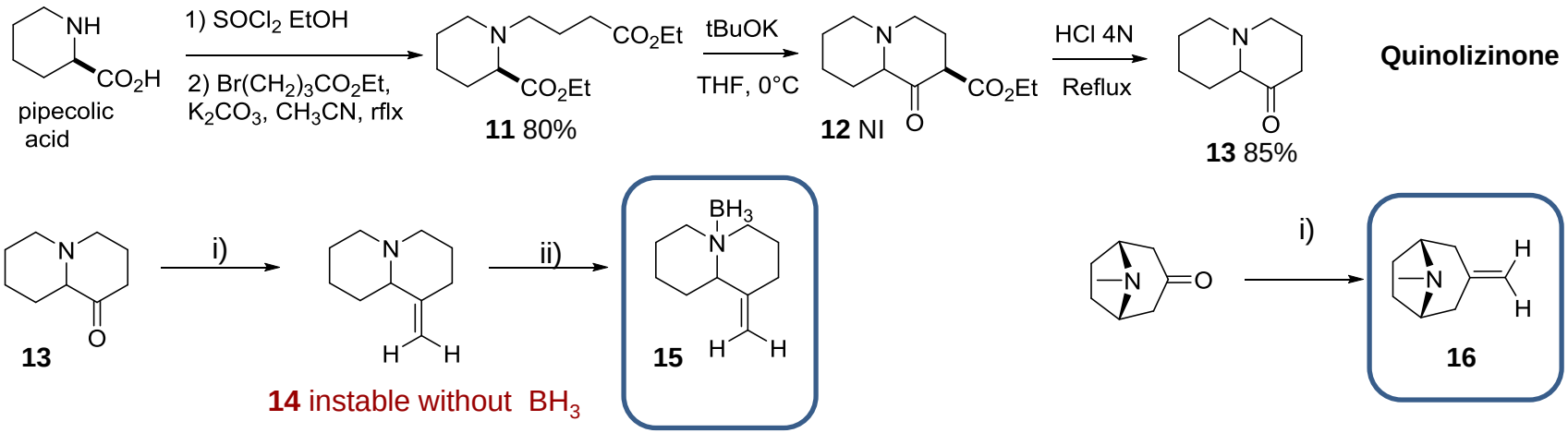
- **The size will be increased using a (Het)Ar + (Het)Ar scafolds**

Results and discussion

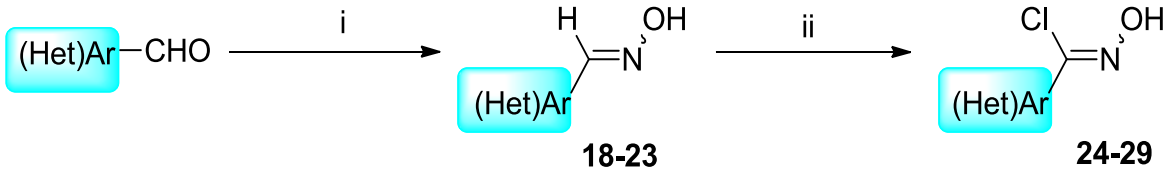
Spiranic series II

Synthesis: *1,3 dipolar addition from an alkene and a chlorooxime*

1) Alkene synthesis (quinolizine and tropane)



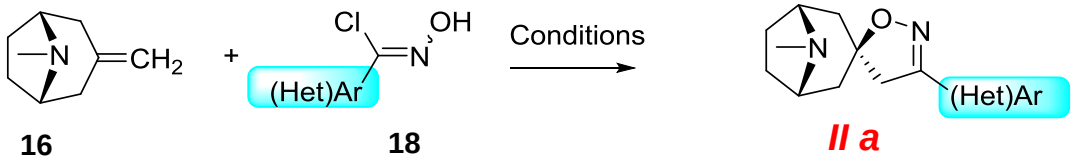
2) Chlorooxime Synthesis



:i) $NH_2OH{\cdot}HCl$ (1.2 eq.), Et_3N (1.5 eq.), CH_2Cl_2, r.t., 12 h; ii) NCS (1.1 eq.), DMF, 0°C to r.t., 12 h.

Results and discussion

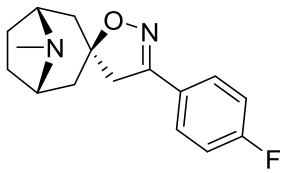
Spiranic series II



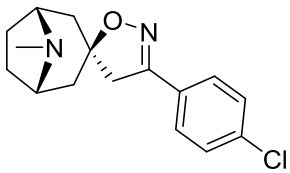
16 **18** ***II a***

1,3 Dipolar cycloaddition

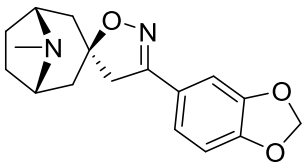
Conditions : chlorooxime **18** (2.0 eq.) $NaHCO_3$ (5.0 eq.), CH_2Cl_2, r.t., 72 h.



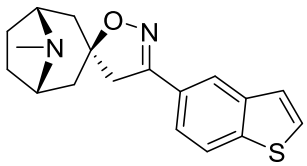
19 (45%)



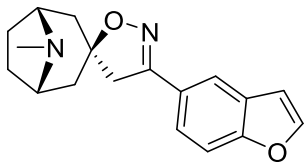
20 (44%)



21 (47%)



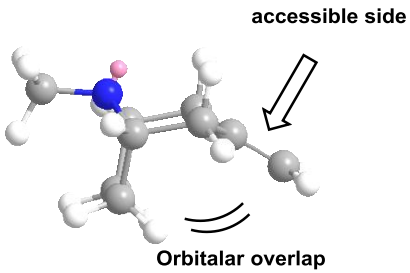
22 (39%)

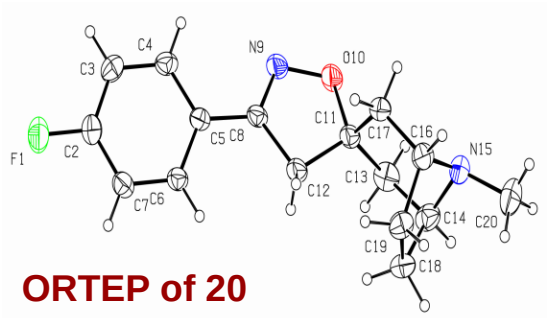


23 (33%)

RX structural proof

Total Regio-selectivity



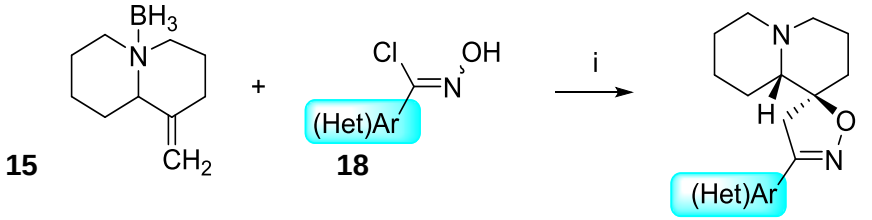


ORTEP of 20

Results and discussion

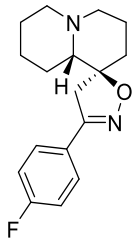
Spiranic series II

Quinolizinic spiro-isoxazoles

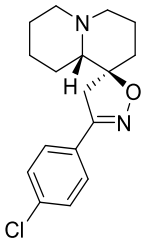


II b

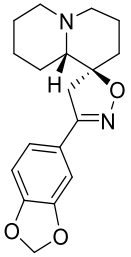
Conditions: chlorooxime **18** (2.0 eq.) $NaHCO_3$ (5.0 eq.), CH_2Cl_2, r.t., 72 h.



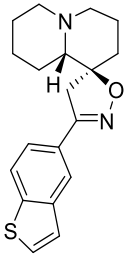
24 (54%)



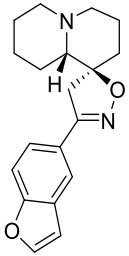
25 (50%)



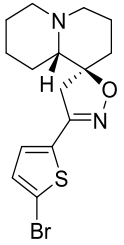
26 (52%)



27 (56%)

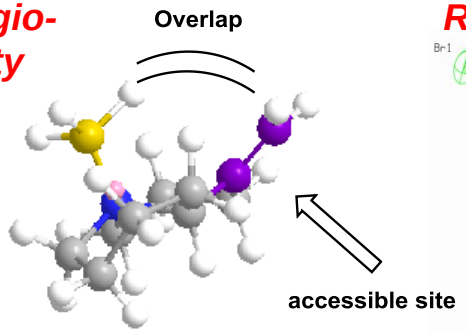


28 (64%)

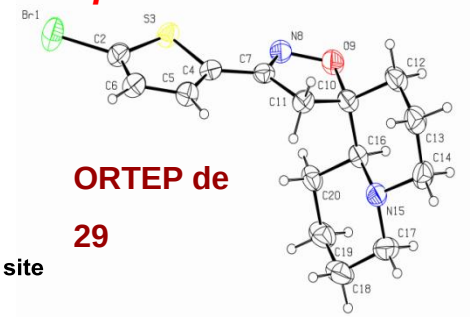


29 (66%)

Total Regio-selectivity



RX proof of 3D structure



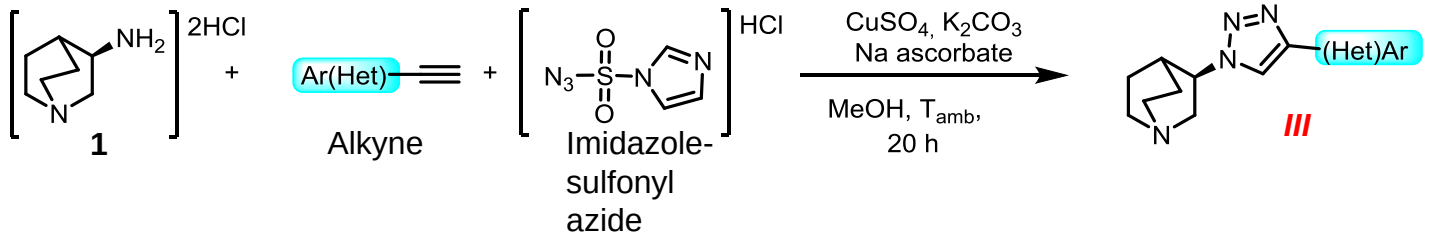
ORTEP de 29

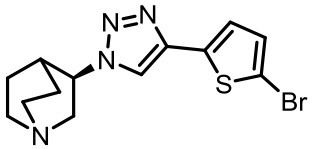
Nevertheless : no affinity detected ….

Results and discussion

Quinuclidine triazole series III

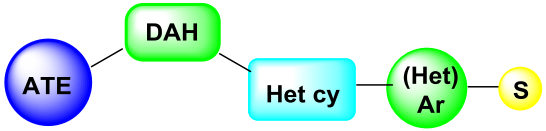
General Synthesis Scheme





38 (42%) **Ki =** 13 nM

Increase
Pharmacophoric model size
Possible ?

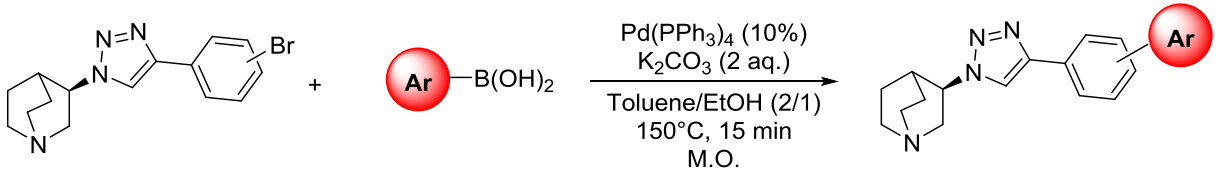


Results and discussion

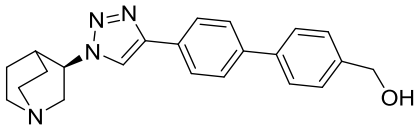
Quinuclidine triazole series III

Ligands size could be increased via a phenyl – (Het)Aryl motif

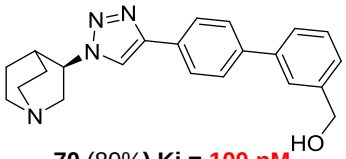
Reaction



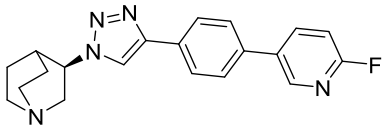
Examples



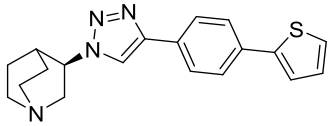
69 (81%) **Ki** = **175 nM**



70 (80%) **Ki** = **100 nM**

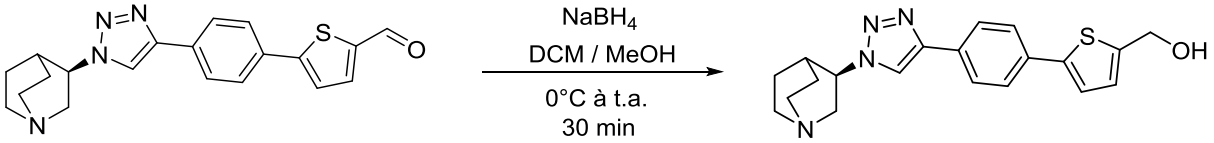


71 (85%) **Ki** = **inactive**



72 (86%) **Ki** = **100 nM**

Reaction



73 (83%) **Ki** = **90 nM**

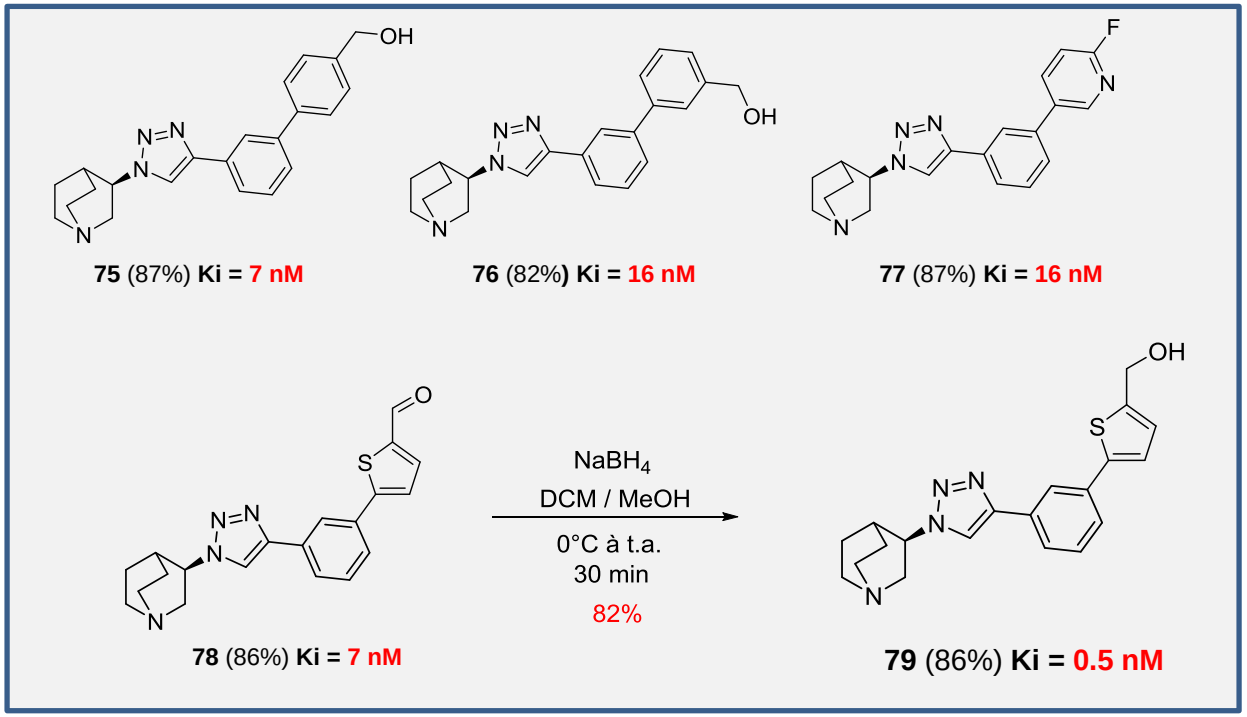
85%

74 **Ki** = **200 nM**

Results and discussion

Quinuclidine triazole series III

Ligands size could be increased via a phenyl – (Het)Aryl motif

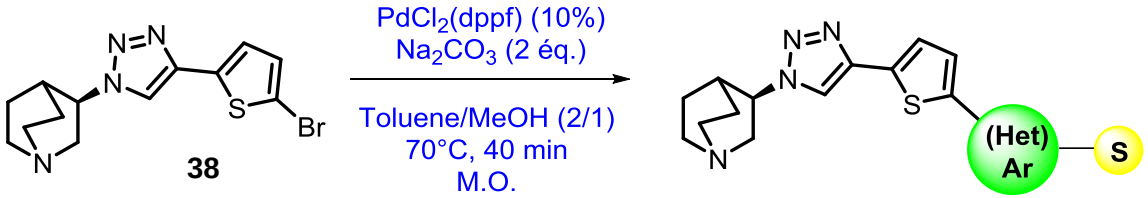


High increase of affinities

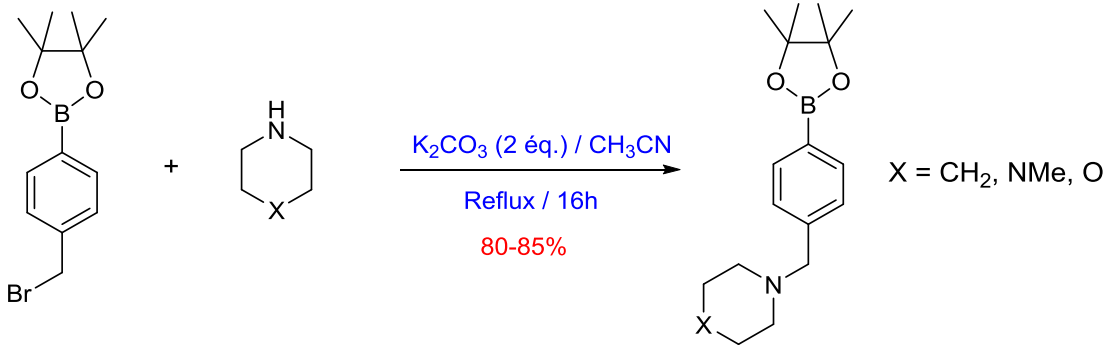
Results and discussion

Quinuclidine triazole series III

Other extension via a brominated thiophene

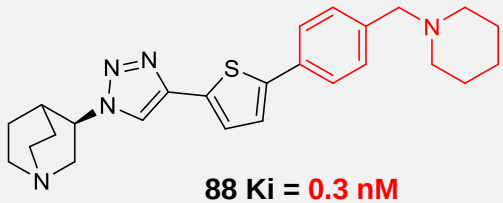


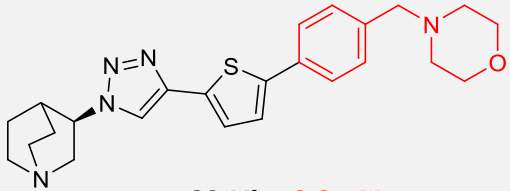
Unknown boron structures

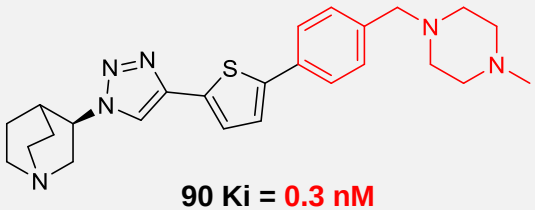


Morpholine
Piperidine
Piperazine

Final Products


88 Ki = 0.3 nM

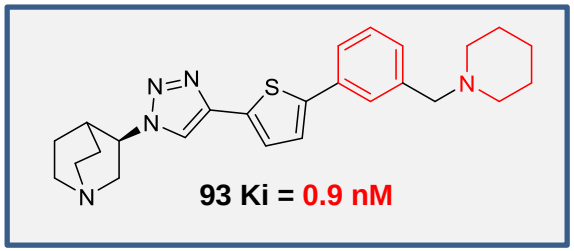

89 Ki = 0.9 nM

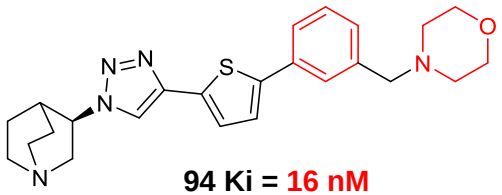

90 Ki = 0.3 nM

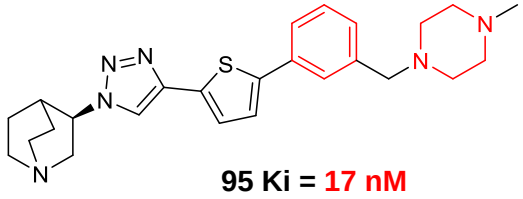
Results and discussion

Quinuclidine triazole series III

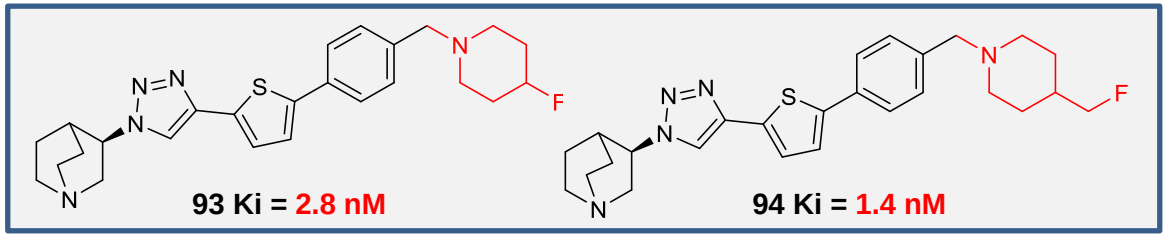
Some Other products

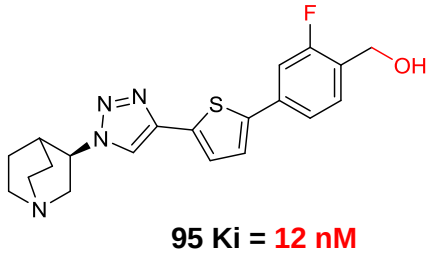


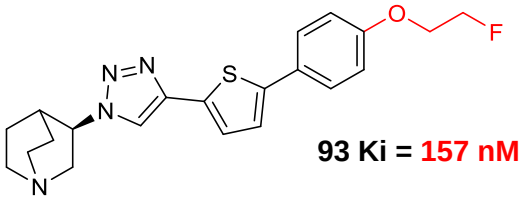


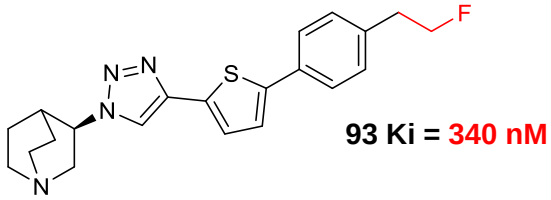


Final fluorinated compounds







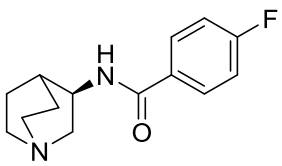


Partial conclusion on the synthesis and SAR studies

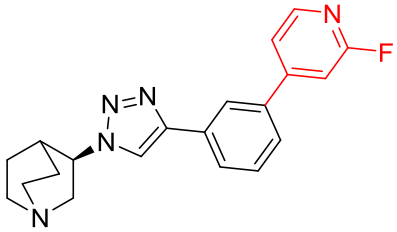
Library Size

	Number of synthesized compounds	>10^{-6} M	1000 to 100 nM	100 to 50 nM	50 to 10 nM	10 to 1 nM	< 1 nM
Triazole Quinuclidine	150	7	22	14	**57**	**37**	**12**

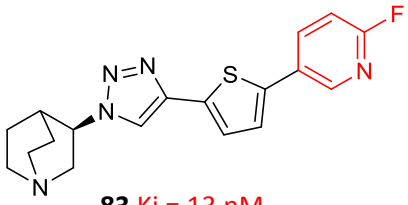
4 fluorinated molecules could be transformed in ^{18}F radioligand by S_N



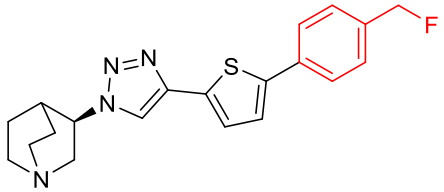
4 Ki = 18 nM



77 Ki = 16 nM



83 Ki = 13 nM



98 Ki = 5 nM

Radiolabelling
Purification (HPLC)
Formulation for in vivo injection

Their presursors are accessible

Results and discussion

Radiolabelling

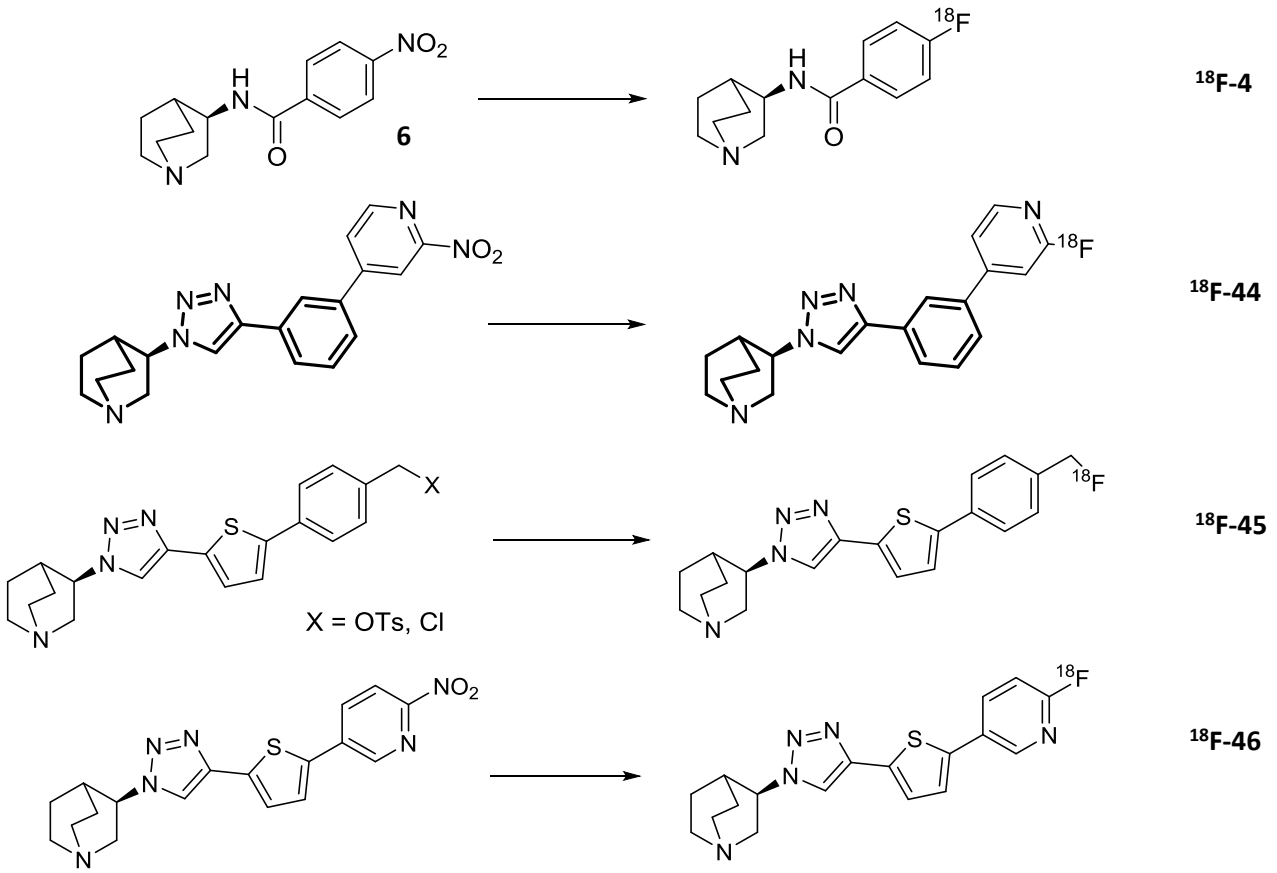
Conditions : [^{18}F]KF, K_{222}, DMSO, microwave 100 W, HPLC

Radiolabeling

Purification

Formulation

Rat Injection

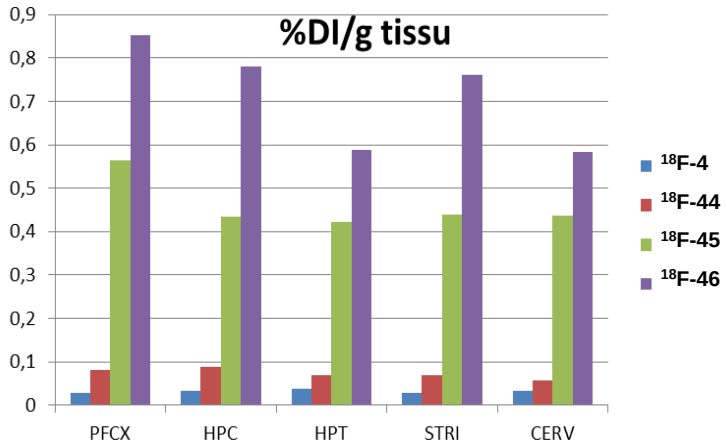


Results and discussion

Rat brain PET imaging

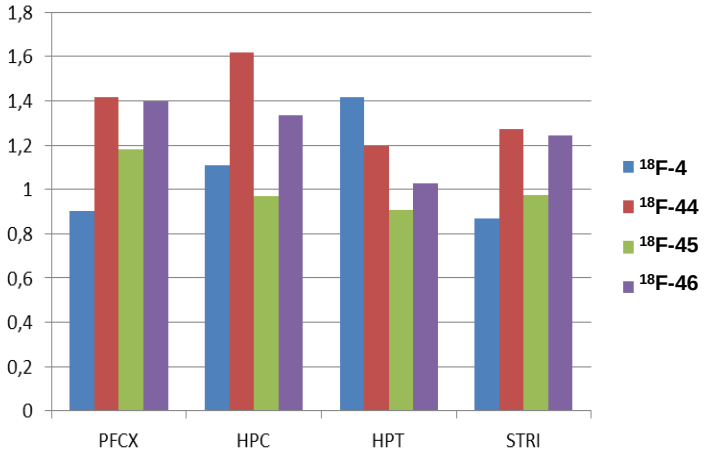
Brain penetration

60 min, i.v.



Brain distribution

60 min, i.v.



- **hippocampus**,
- cortex,
- **cerebellum**,
- olfactory bulb,
- **striatum**,
- thalamus,
- spinal cord

In vitro Reference **4** is not brain available

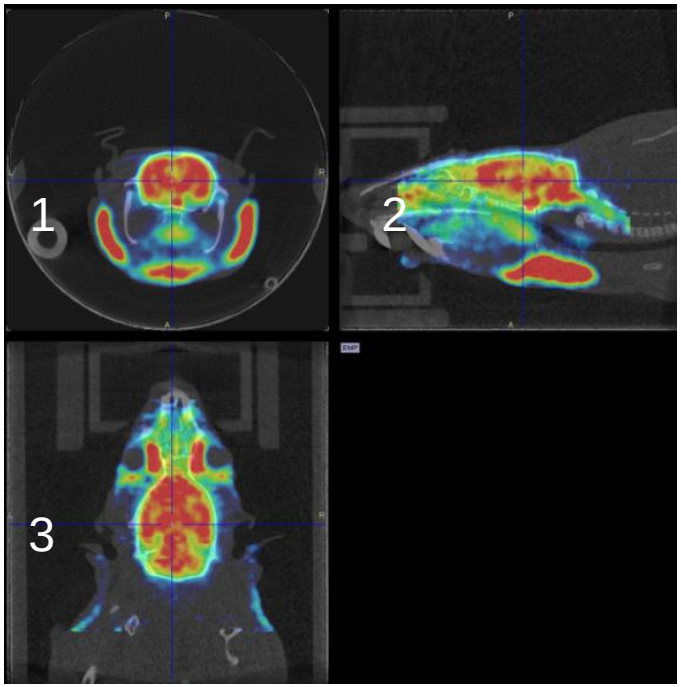
Best compounds are ^{18}F **45 and 46**

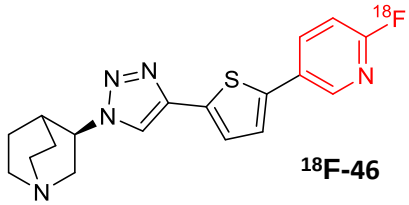
Alpha 7 receptor zones accumulation

but distribution appears as unselective

Results and discussion

Rat brain PET imaging





^{18}F-46

46 Ki = 13 nM

With a fine tune of the signals intensity

Details are perceptible

Repartition is not fully homogeneous

A relative specificity appeared

Other radiolabelled compound are currently in evaluation

Conclusions

Novel and original alpha 7 receptor ligands are developed

A WO patent is filed

Structures of probes were optimized following a medicinal chemistry program

This series are able to furnish ^{18}F ligands with excellent affinity constants

The radiollabeling is reproductible, product are stable enough to envision their use in other studies

Number of tracers is actually incremented in order to find the best brain selective agent.

The final molecules remain agonists (not shown) despite their size and molecular weight

We proved the high potency of these series

Publications and other production on this subject see http://www.icoa.fr/fr/recherche/productions-scientifiques

Acknowledgments

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Dr Frédéric Pin (synth)
Dr Aziz Ouach (synth)
Dr Emilie Bertrand (synth)
Zuhal Gulhan (in vitro)
Gabrielle Chicheri (^{18}F)
Dr Sylvie Mavel (mod mol)

Scientific participants:

Pr Sylvain Routier, chemistry
Pr Franck Suzenet, chemistry
Dr Sylvie Chalon, neurobiology
Dr Johnny Vercouillie, radiochemistry
Pr Denis Guilloteau, clinical radiopharmacy
Dr Jean-Bernard Deloye, Cyclopharma SA

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Labex IRON (ANR-11-LABX-0018-01***)***