Steps in PET tracer development

VUmc

- Selection of lead compounds
- Synthesis of target compounds
- Optimization of pharmacokinetic properties
- Radiolabeling with suitable radionuclide
- Binding characteristics on cells and membranes or autoradiography
- Small animal: ex vivo biodistribution, (*in vitro*, *ex vivo*) autoradiography
- Distribution studies in mice/rats with preclinical PET imaging
- Metabolism studies (N=12)
- Toxicological safety assessment. (microdosing concept)
- Set up GMP production
- Validation of PET tracer in human volunteers, including dosimetry studies, Typically N=6-12
- Proof of concept: 10 patients vs 10 controls
- Suited for clinical applications

VUmc

Complicated stuff, best to discuss with a case:

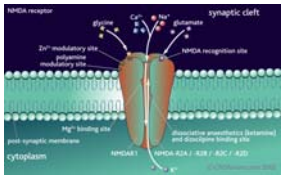
development of [^{18}F]PK209, a NMDA PET tracer

Introduction

VUmc

Many nervous system disorders are associated with *N*-methyl-D-aspartate receptor (NMDAR)

Subtype of the glutamate receptor family, contributes to many processes.
- Physiological (development, learning and memory)
- Pathological:
  - Neurodegenerative disorders, eg Alzheimer's, Huntington
  - Psychiatric disorders, eg Schizophrenia



Kotermanski, J. Physiol 2009, 4589-4603
Kew, Psychopharmacology 2005, 179, 4-29
Bordji, Rev Neurosciences 2011, 22, 285-294
Furukawa, Nature 2005, 438, 185-192
Kalia, Lancet Neurology 2008, 7, 742-755

4

Aim

VUmc

***In vivo* imaging of the NMDA receptor using PET**

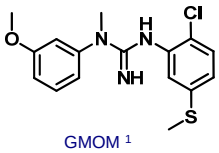
At the start of the program no suitable PET tracer available

New compounds to be designed from literature leads
synthesized and evaluated *in vitro*
high affinity compounds will be radiolabelled with ^{11}C or ^{18}F
evaluation of labelled compounds *in vivo* in animals

Meanwhile: [^{18}F]GE179, published last March in J Nucl Med.
Good brain uptake, homogenous distribution in the brain as can be expected.
However not conclusive yet about the specificity of the PET signal.

Lead compound

VUmc



GMOM [1]

- K_i: 5.2 nM ([^{3}H]MK-801) [1]
- LogP: 2.86 [1]

[1] Waterhouse *et al.* Nucl Med & Biol (2004) 939 and Dumont *et al*, Bioorg. Med Chem Lett (2002) 1883

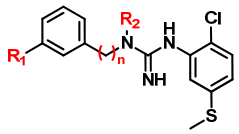
Strategy

VUmc

Guanidine structure of GMOM as template used for a new set of ion channel blockers

Possible modifications:

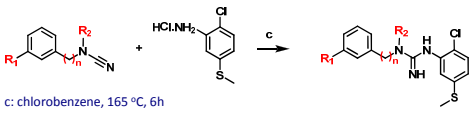
- introduction of 1 or 2 methylene group(s) (n)
- introduction of fluoro-alkoxy chains (R_1)
- hydrogen or methyl group on guanidine (R_2)



7

Chemistry (step 2)

VUmc



c: chlorobenzene, 165 °C, 6h

R_1 =	n =	R_2 =
H	0, 1, 2	H, Me
O-Me	0, 1, 2	H, Me
OH	0	Me
$O\text{-}CHF_2$	0	Me
$O\text{-}CF_3$	0	Me

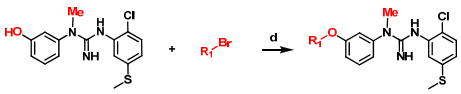
Total of 15 compounds

Yields: 33 - 75%

8

Chemistry (fluoro-alkanes)

VUmc



d: $Br(CH_2)_{1\text{-}3}F$, potassium carbonate, potassium iodide, DMF

R_1 =
CH_2F
CH_2CH_2F
$CH_2CH_2CH_2F$

additional 3 compounds

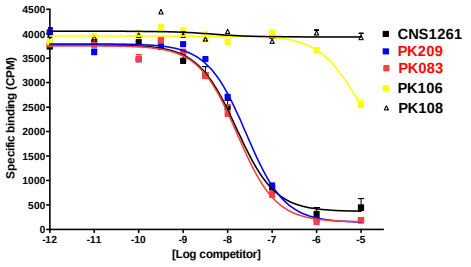
Yield: 61 - 73%

9

In vitro binding assay

VUmc

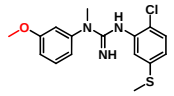
Rat brain frontal cortex homogenates, with [^{3}H]MK801



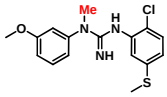
10

In vitro affinities

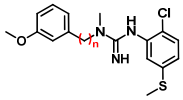
VUmc



Methoxy is essential



Methyl is essential



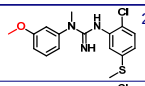
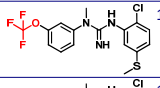
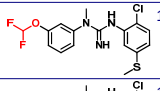
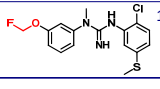
Elongation of alkane: reduced affinities

11

In vitro affinities

VUmc

Influence of fluorine substitution in methoxy moiety

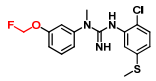
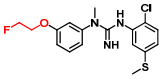
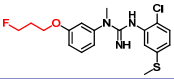
$R_1 = OCH_3$		22 nM
$R_1 = OCF_3$		12 nM
$R_1 = OCHF_2$		10 nM
$R_1 = OCH_2F$		18 nM

12

In vitro affinities

VUmc

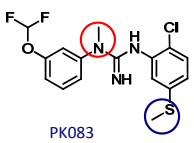
Fluoroalkanes, elongation not allowed

R1 = OCH_2F		18 nM
R1 = $O(CH_2)_2F$		155 nM
R1 = $O(CH_2)_3F$		179 nM

13

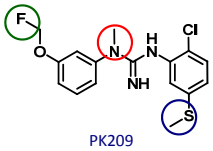
Selected compounds

VUmc



PK083

K_i: 10 nM



PK209

K_i: 18 nM

Options for radiolabeling:

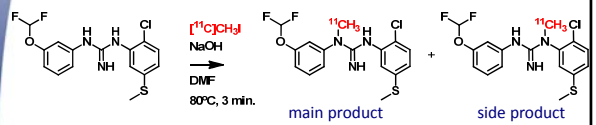
N-Methyl (carbon-11)

S-Methyl (carbon-11)

Fluoromethoxy (fluor-18)

Radiolabelling [^{11}C]PK083

VUmc

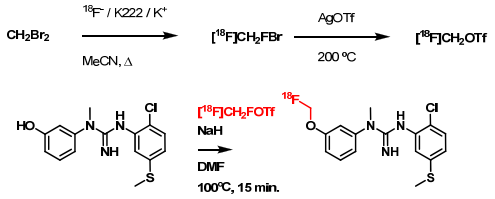


Base	µmol	Solvent (300 µL)	RCY (HPLC)	RCY (Formulated)	Radiochemical purity
NaOH, 5M	25	DMF	51 ± 2%	9 - 26%	> 97.5 %

n = 7, decay corrected

15

Radiolabelling [^{18}F]PK209

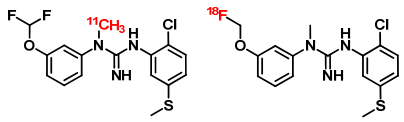


Base	µmol	Solvent (300 µL)	RCY (HPLC)	RCY (Formulated)	Radiochemical purity
NaH	50	DMF	ND	8 - 25%	> 96 %

n = 6, decay corrected

16

Specific activity and $LogD_{7.4}$



Specific activity	>65 GBq/µmol	>57 GBq/µmol
$LogD_{7.4,oct}$	1.76 ± 0.01	1.45 ± 0.02

17

Metabolite analysis – Methods

- Healthy male Wistar rats were injected with 90-100 MBq of tracer
- Blood samples were obtained and brains were taken out at 15 and 45 minutes post injection
- Blood plasma was passed over SPE cartridge; non-polar fraction was then eluted with MeOH and analysed by HPLC (incl. offline detection)
- Brains were homogenised in H_2O, centrifuged and the supernatant was passed over a SPE cartridge; non-polar fraction was then eluted with MeOH and analysed by HPLC (incl. offline detection)

18

In vivo metabolism in male wistar rats

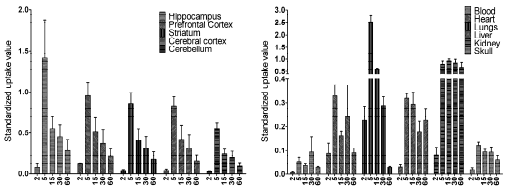
	Time (min)	N	Polar fraction	Nonpolar fraction		
				Parent	Metabolite	Sep-Pak
[^{11}C]PK083						
Brain	5	3	3(2)	74 (28)	20(21)	4(5)
	15	3	4(4)	73 (31)	21(23)	3(3)
	30	4	7(3)	63 (20)	24(14)	6(9)
	60	4	11(5)	54 (19)	29(10)	6(6)
Plasma	5	4	17(4)	39 (20)	43(15)	3(2)
	15	4	29(11)	17 (3)	53(13)	2(1)
	30	4	25(9)	14 (7)	61(6)	2(1)
	60	4	34(7)	7 (2)	59(6)	2(0)
[^{18}F]PK209						
Brain	5	3	3(2)	86 (9)	8(9)	2(2)
	15	4	2(1)	72 (17)	24(20)	4(4)
	30	3	3(3)	76 (15)	18(12)	3(1)
	60	4	4(1)	70 (16)	25(14)	1(1)
Plasma	5	4	7(6)	38 (6)	48(11)	7(8)
	15	3	20(2)	25 (6)	54(8)	2(1)
	30	4	16(13)	21 (14)	59(14)	5(6)
	60	3	29(0)	9 (4)	61(3)	1(0)

Ex vivo biodistribution – Methods

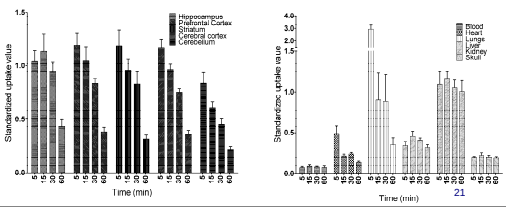
- Healthy male B6C3 mice were injected with 50 MBq of tracer
- Mice were sacrificed at different time points after injection
  - (2), 5, 15, 30 and 60 min p.i.
- Organs and brain sections were collected, weighed and counted in gammacounter
- Data were expressed as injected dose per gram (%ID/g)

Ex vivo biodistribution in B6C3 mice

[^{11}C]PK083

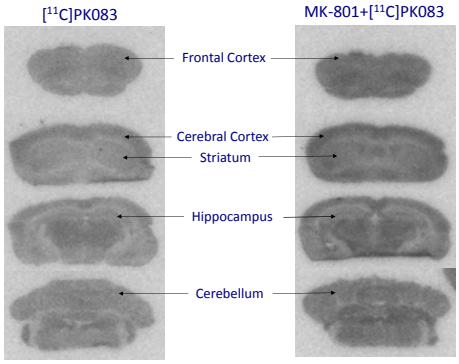


[^{18}F]PK209



Ex vivo autoradiography (rats)

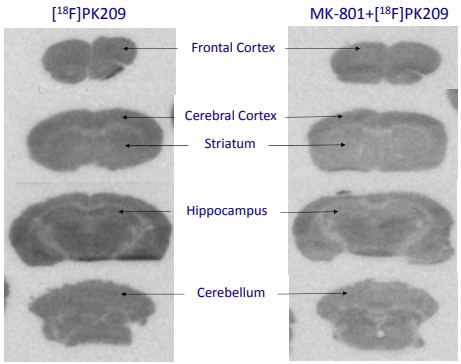
VUmc



22

Ex vivo autoradiography (rats)

VUmc



23

Pharmacological selectivity profile

VUmc

- 10 µM PK209 showed no significant binding to 70 of 79 targets tested
- 50-fold selectivity for NMDAr ion-channel over the other 8 targets

	Target	IC_{50} (M)	Ki (M)	nH
1	Adrenergic alpha 1A	$6.2 \cdot 10^{-6}$	$3.1 \cdot 10^{-6}$	1.1
2	Muscarinic M1	$5.3 \cdot 10^{-6}$	$4.6 \cdot 10^{-6}$	1.0
3	Muscarinic M2	$4.2 \cdot 10^{-6}$	$2.9 \cdot 10^{-6}$	0.8
4	Opioid κ (KOP)	$7.5 \cdot 10^{-6}$	$5.0 \cdot 10^{-6}$	1.0
5	Opioid µ (MOP)	$2.8 \cdot 10^{-6}$	$1.2 \cdot 10^{-6}$	0.8
6	NMDA (PCP site)	$4.0 \cdot 10^{-8}$	$2.2 \cdot 10^{-8}$	1.5
7	sigma (1 and 2)	$2.2 \cdot 10^{-6}$	$1.8 \cdot 10^{-6}$	0.9
8	Ca^{2+} channel (L, verapamil site)	$4.1 \cdot 10^{-6}$	$2.0 \cdot 10^{-6}$	0.7
9	Na^{+} channel (site 2)	$5.2 \cdot 10^{-6}$	$4.6 \cdot 10^{-6}$	1.0

24

[^{18}F]PK209 PET study in rhesus monkey

Subjects: 3 male rhesus monkeys, aged 17-19 years, weight: 7.6-13.5 kg

Sedation with midazolam (0.3 mg/kg) + medetomidine (60 µg/kg), anaesthetised with propofol (0.2 mg/kg/min)

T1-weighted structural MRI scans in 3T Siemens Trio scanner

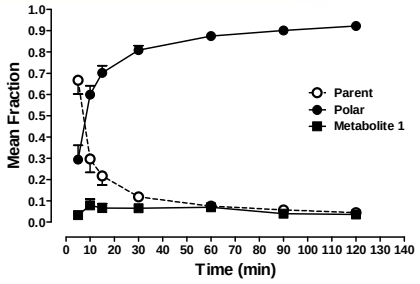
120 min dynamic PET scans in the ECAT HRRT scanner

Continuous and discrete manual blood samples from the femoral artery

Blocking scans performed 1-2 weeks after baseline. NMDArs blocked with MK-801 (0.3 mg/kg), administered 30 min before radiotracer injection

25

Plasma metabolism in rhesus monkey

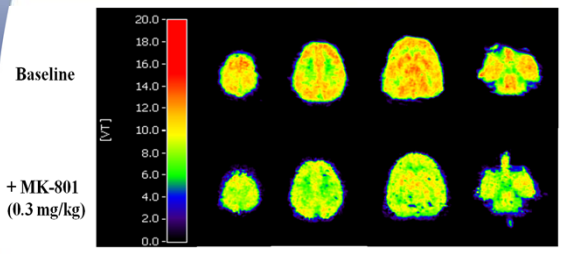


No effect of MK-801 on metabolism, no defluorination

[^{18}F]PK209 shows fast metabolism in plasma, comparable to rats.

26

Logan V_T parametric images, subject 1

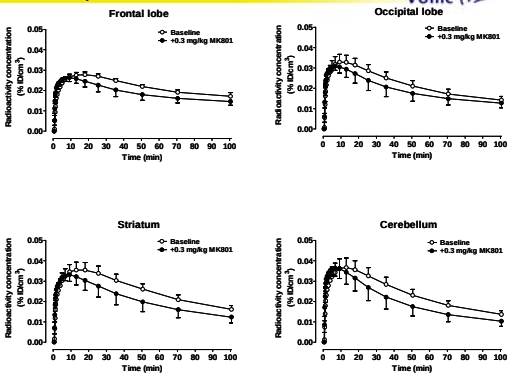


Images presented through the transaxial plane

Smoothed by isotropic Gaussian filter of 2 mm at full-width, half maximum

27

Time-activity curves



Significant decrease in uptake following MK-801 administration

28

Microdosing toxicity

Performed at Advinus, India according to EMEA/CPMP/SWP/2599/02/Rev 1, June 2004 and EMEA/CPMP/ICH/286/95 June 2009

Wistar rats, 1 and 14 day toxicity at a dose of 95 µg/kg (1000 times the expected tracerdose)

5 males, 5 females per time point, vehicle control groups

clinical signs, mortality, changes in body weight and food intake, terminal hematology, coagulation and clinical chemistry, organ weights, gross pathology and histopathological examination of liver and lungs.

Results: no toxicity observed.

29

Conclusions

2 compounds were selected for radiolabeling
From a series of NMDA noncompetitive ion channel blockers,

Reliable radiosynthesis was established

Ex vivo autoradiography showed unfavorable results with [^{11}C]PK083

[^{18}F]PK209 PET in rhesus monkey showed modest reduction of the signal after MK-801 administration.

PK209 was shown to be safe in a microdosing toxicity study

Further characterisation in humans is warranted

30

Acknowledgements

Chemistry: Pieter Klein & Hans Christiaans
Pharmacology: Thanos Metaxas
Modelling: Sandeep Golla

PI: Bart van Berckel
Supervision: Ronald Boellaard & Adriaan Lammertsma

This research was supported by the Center for Translational Molecular Medicine, CTMM