



^{18}F -MARKIERTE PHENYLPIPERAZINE ALS NEUE STRUKTURMOTIVE FÜR SPEZIFISCHE DOPAMIN D_4 -REZEPTORLIGANDEN

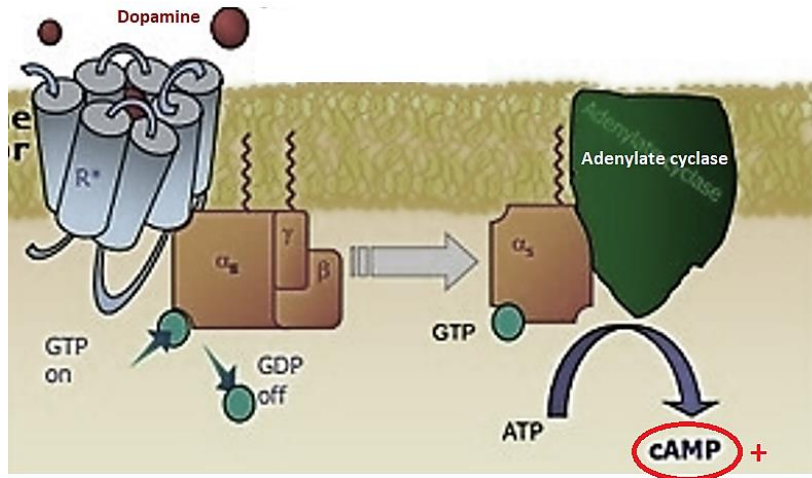
05.04.2019 | Michael Willmann

Forschungszentrum Jülich

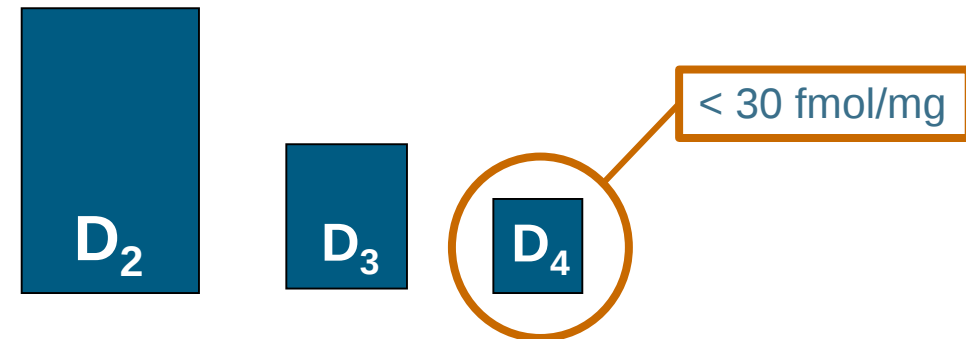
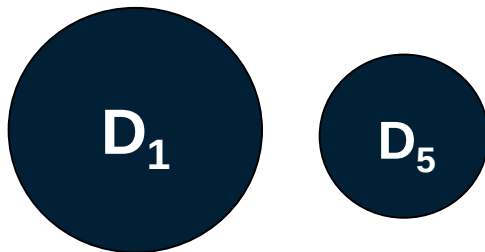
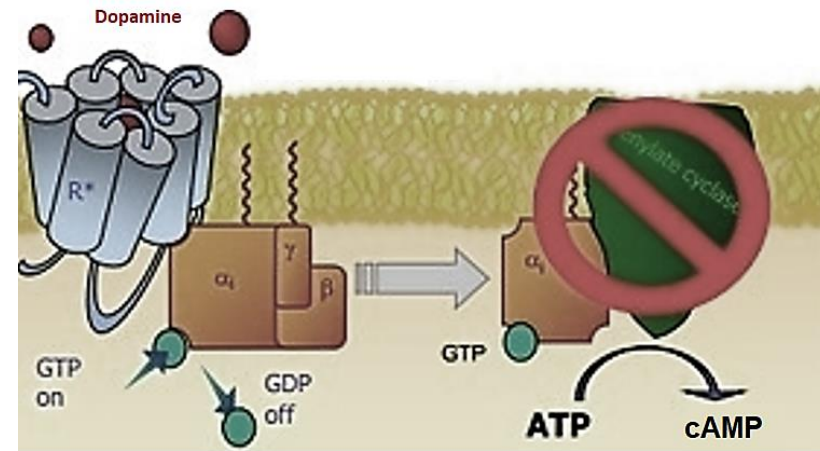
Institut für Neurowissenschaften und Medizin (INM-5)

DOPAMIN-REZEPTOR-SUBTYPEN

D₁-Familie:



D₂-Familie:



WARUM DER D₄-REZEPTOR?

„Terra incognita“

Clozapin: Erstes atypisches Neuroleptikum (1972):

- D₄-selektiv
- Kein EPS* als Nebenwirkung

*Extrapyramydalmotorisches Syndrom

Aktuelles Interesse am D₄-Rezeptor (ab 1998):^[1]

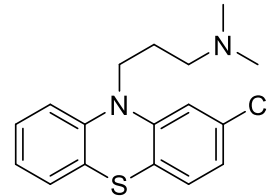
ADHS^[1]

Sucht (Kokain, Amphetamine)^[2]

Libido^[3]

Parkinson^[1]

Tumorthherapie (Glioblastoma)^[4]



Chlorpromazin

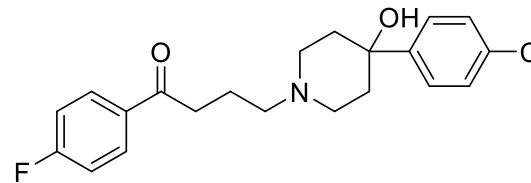
$K_i(D_1)$ 90 nM

$K_i(D_2)$ 3 nM

$K_i(D_3)$ 4 nM

$K_i(D_4)$ 35 nM

$K_i(D_5)$ 130 nM



Haloperidol

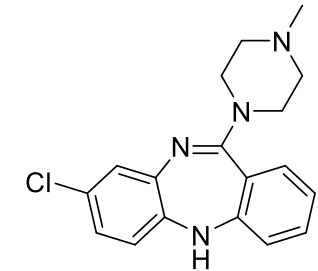
$K_i(D_1)$ 80 nM

$K_i(D_2)$ 1.2 nM

$K_i(D_3)$ 7 nM

$K_i(D_4)$ 2.3 nM

$K_i(D_5)$ 100 nM



Clozapin

$K_i(D_1)$ 170 nM

$K_i(D_2)$ 230 nM

$K_i(D_3)$ 170 nM

$K_i(D_4)$ 21 nM

$K_i(D_5)$ 330 nM

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- Libido
- Parkinson
- Tumorthherapie (Glioblastoma)
- Sucht (Kokain, Amphetamine)

1998

Evidence that the dopamine D₄ receptor is a susceptibility gene in attention deficit hyperactivity disorder

SL Smalley, JN Bailey, CG Palmer,
DB Cantwell, JJ McGough, MA Del'Homme

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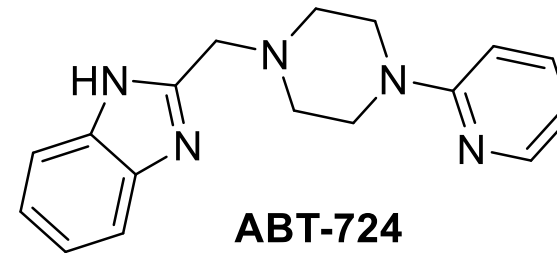
- ADHS
 - **Libido**
- Parkinson
- Tumorthherapie (Glioblastoma)
- Sucht (Kokain, Amphetamine)

2004

Activation of dopamine D₄ receptors by ABT-724 induces penile erection in rats

Jorge D. Brioni^{**†}, Robert B. Moreland^{*}, Marlon Cowart^{*}, Gin C. Hsieh^{*}, Andrew O. Stewart^{*}, Petter Hedlund[†], Diana L. Donnelly-Roberts^{*}, Masaki Nakane^{*}, James J. Lynch III^{*}, Teodozyi Kolasa^{*}, James S. Polakowski^{*}, Mark A. Osinski^{*}, Kennan Marsh^{*}, Karl-Erik Andersson[†], and James P. Sullivan^{*}

^{*}Neuroscience Research, Global Pharmaceutical Research and Development, Abbott Laboratories, Abbott Park, IL 60064; and [†]Department of Clinical Pharmacology, Lund University Hospital, S-22185 Lund, Sweden



WARUM DER D₄-REZEPTOR?

„Terra incognita“

2012

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- Libido
 - **Parkinson**
- Tumorthherapie (Glioblastoma)
- Sucht (Kokain, Amphetamine)

L-745,870 Reduces L-DOPA-Induced Dyskinesia in the 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine-Lesioned Macaque Model of Parkinson's Disease

Philippe Huot, Tom H. Johnston, James B. Koprach, Ahmed Aman, Susan H. Fox, and Jonathan M. Brotchie

Toronto Western Research Institute (P.H., T.H.J., J.B.K., S.H.F., J.M.B.) Division of Neurology, Movement Disorder Clinic, Toronto Western Hospital (P.H., S.H.F.), University Health Network, Toronto, Ontario, Canada; Atuka Inc., Toronto, Ontario, Canada (T.H.J., J.B.K., J.M.B.); and Ontario Institute for Cancer Research, Toronto, Ontario, Canada (A.A.)

Received April 16, 2012; accepted **May 21, 2012**

WARUM DER D₄-REZEPTOR?

„Terra incognita“

2016

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Cancer Cell 2016 June 13; 29(6): 859–873. doi:10.1016/j.ccell.2016.05.002.

Inhibition of Dopamine Receptor D₄ Impedes Autophagic Flux, Proliferation, and Survival of Glioblastoma Stem Cells

Sonam Dolma^{1,2}, Hayden J. Selvadurai¹, Xiaoyang Lan^{1,3}, Lillian Lee¹, Michelle Kushida¹, Veronique Voisin⁵, Heather Whetstone¹, Milly So¹, Tzvi Aviv¹, Nicole Park^{1,3}, Xueming Zhu¹, ChangJiang Xu⁵, Renee Head¹, Katherine J. Rowland¹, Mark Bernstein⁶, Ian D. Clarke^{1,7}, Gary Bader^{3,5}, Lea Harrington⁸, John H. Brumell^{3,9}, Mike Tyers⁸, and Peter B. Dirks^{1,2,3,4,*}

WARUM DER D₄-REZEPTOR?

„Terra incognita“

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- ADHS
- Libido
- Parkinson
- Tumorthherapie (Glioblastoma)
 - Sucht (Kokain, Amphetamine)

2017

Dopamine receptor D₄ promoter hypermethylation increases the risk of drug addiction

HUIHUI JI^{1*}, XUTING XU^{1*}, GUILI LIU^{1*}, HUIFEN LIU^{2*}, QINWEN WANG¹, WENWEN SHEN², LONGHUI LI², XIAOHU XIE², HAOCHANG HU¹, LEI XU¹, WENHUA ZHOU² and SHIWEI DUAN¹

¹Medical Genetics Center, School of Medicine, Ningbo University, Ningbo, Zhejiang 315211; ²Laboratory of Behavioral Neuroscience, Ningbo Addiction Research and Treatment Center, Ningbo, Zhejiang 315010, P.R. China

Received May 4, 2016; Accepted April 10, 2017



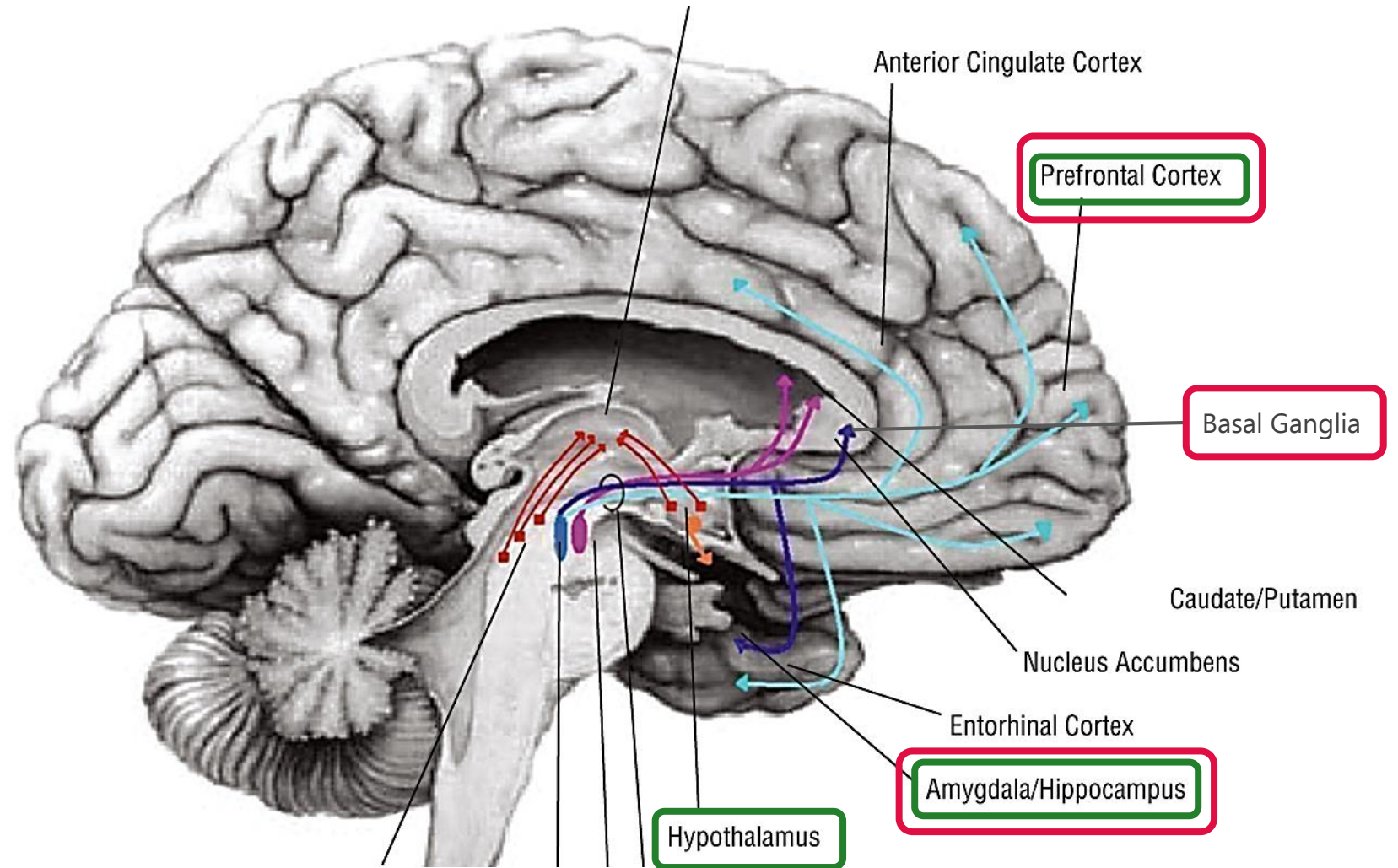
D₄-Rezeptorligand als „Goldstandard“ essentiell für tiefergehendes Verständnis

DOPAMIN D₄-REZEPTOR

Lokalisation im Gehirn

in situ Hybridisierung

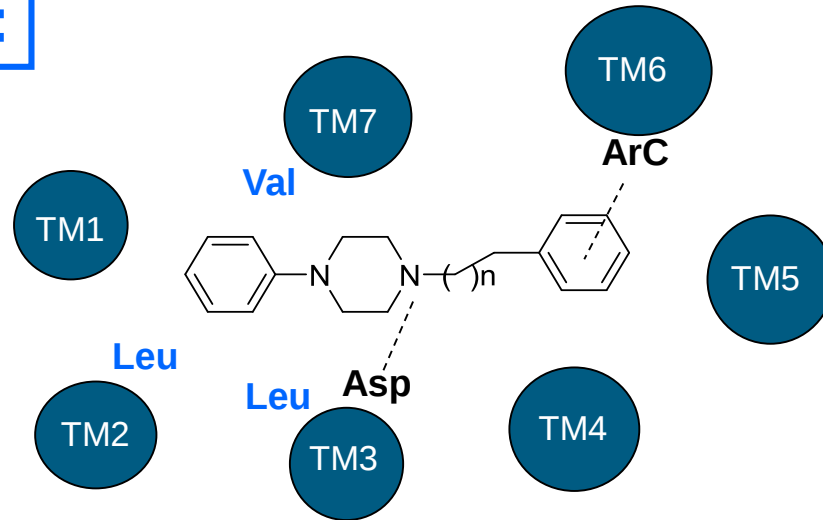
Substraktive
Methoden



„STRUCTURE AFFINITY RELATIONSHIPS“ (SAFIR)

Worauf beruhen Affinität und Selektivität?

D₄R:

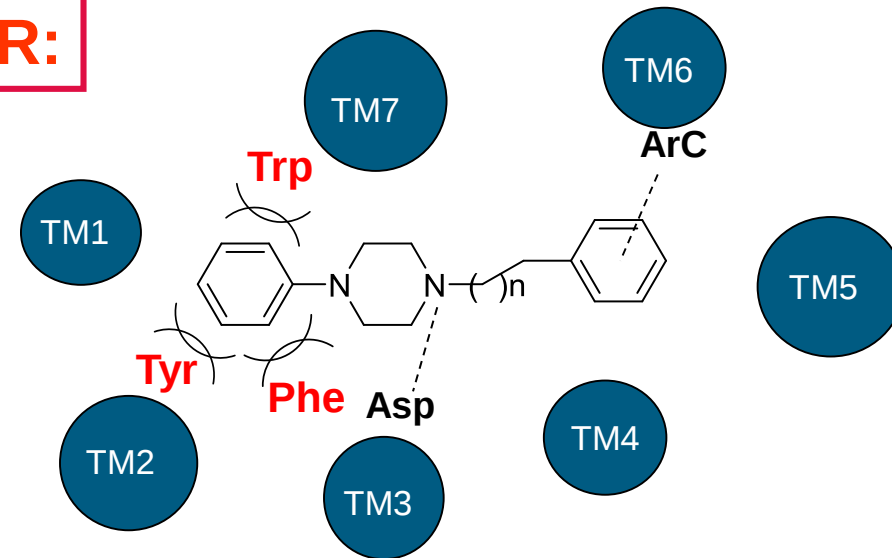


Affinität:

ArC Aromatic Complex
Asp -CH₂COOH

*TM 1-7: Transmembran Helices

D₂R:



Selektivität:

D₂R:

Trp
Phe
Tyr

-CH₂C₈H₆N
-CH₂C₆H₅
-CH₂C₆H₄OH

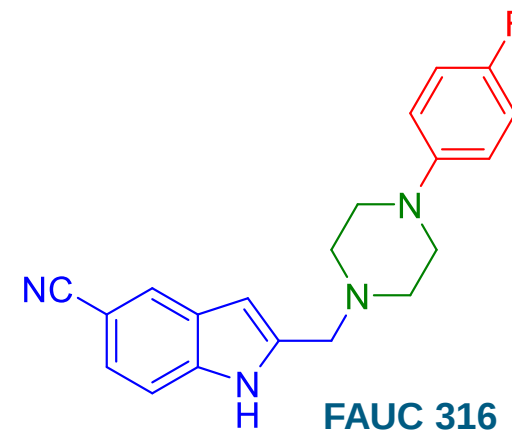
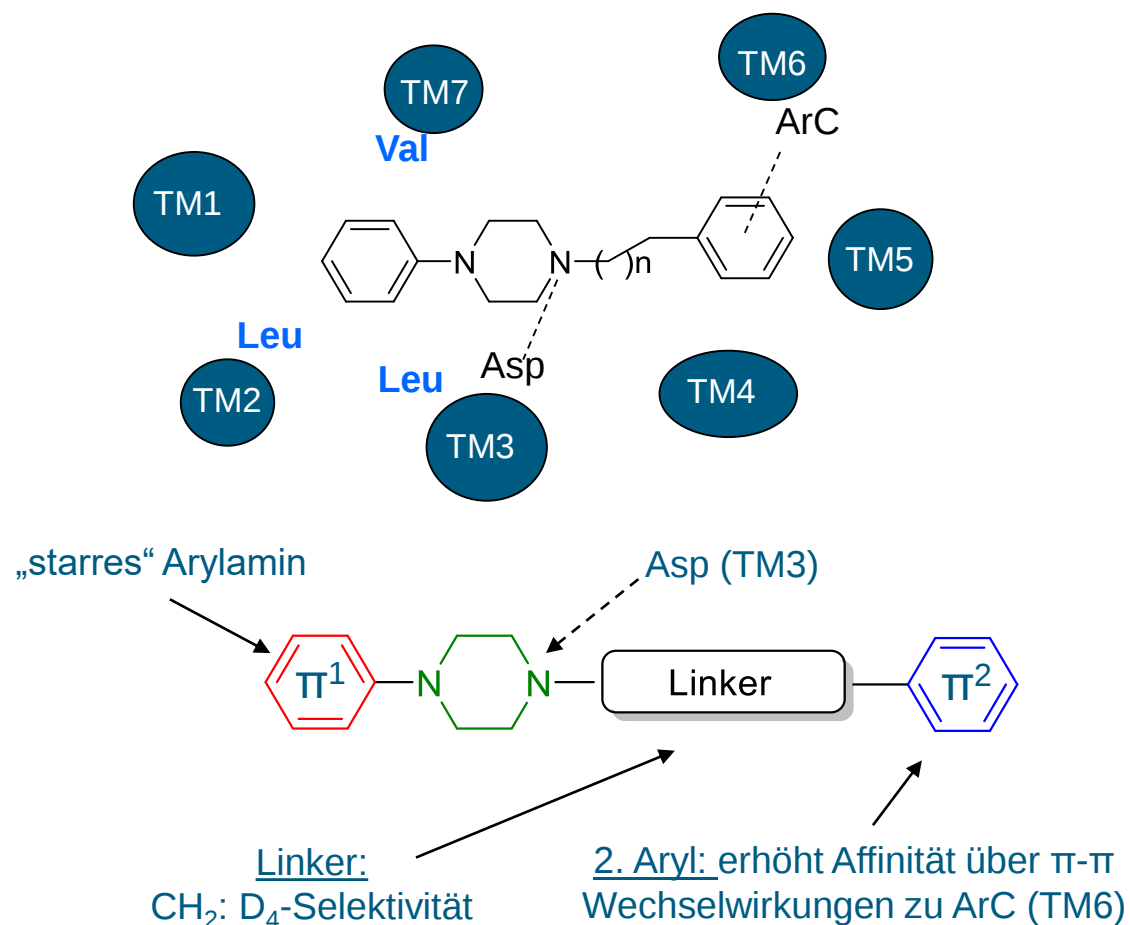
D₄R:

Leu
Val

-CH₂CH₂(CH₃)₂
-CH₂(CH₃)₂

„STRUCTURE AFFINITY RELATIONSHIPS“ (SAFIR)

Abgeleitete Leitstrukturen

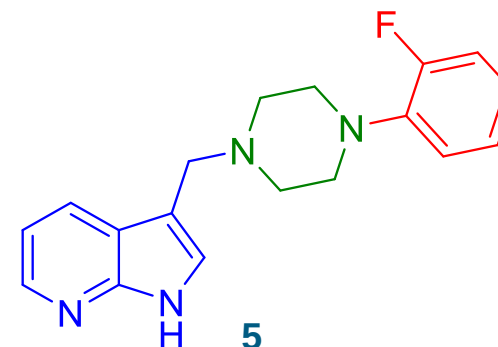


$K_i(D_2) > 19000 \text{ nM}$
 $K_i(D_3) > 15000 \text{ nM}$
 $K_i(D_4) = 1 \text{ nM}$

cLogP = 3,54

In vitro Autoradiographien:^[1]

- nicht-spezifische Bindung
- zu lipophil

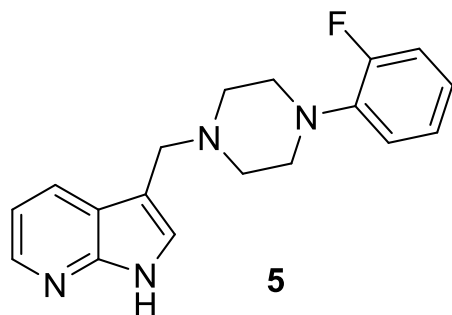


$K_i(D_2) > 5000 \text{ nM}$
 $K_i(D_3) > 2500 \text{ nM}$
 $K_i(D_4) = 3,11 \text{ nM}$

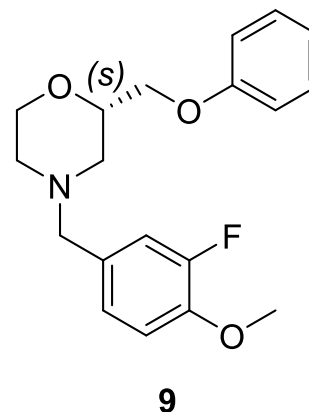
cLogP = 3,14

- vergleichsweise Hydrophil

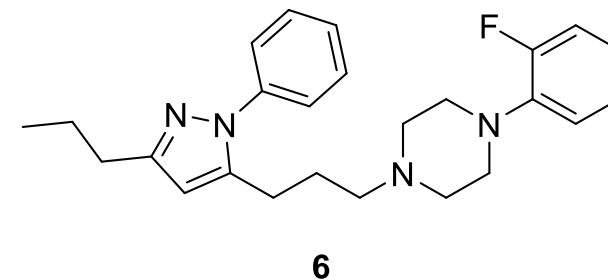
AUSGEWÄHLTE D₄-REZEPTORLIGANDEN



$K_i(D_2) > 5000 \text{ nM}$
 $K_i(D_3) 2900 \pm 151 \text{ nM}$
 $K_i(D_4) \mathbf{3,11} \pm 0,85 \text{ nM}$
 $cLog P 3,14$



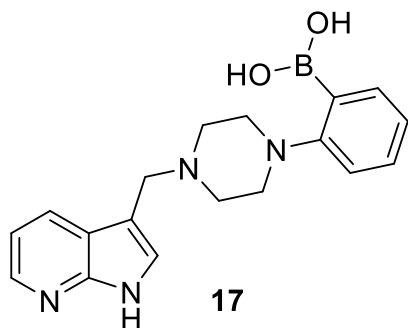
$K_i(D_4) \mathbf{14,3} \text{ nM}$
 $cLog P 3,18$



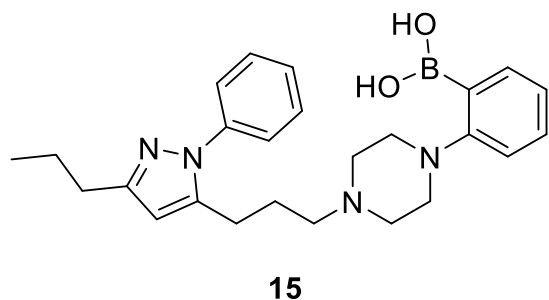
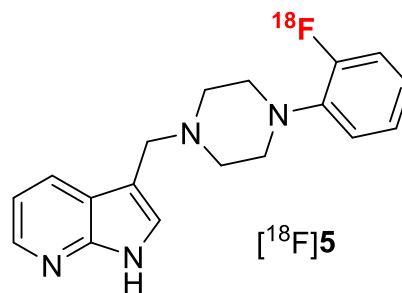
$IC_{50}(D_2) 696 \text{ nM}$
 $IC_{50}(D_3) 87 \text{ nM}$
 $IC_{50}(D_4) \mathbf{3,5} \text{ nM}$

RADIOFLUORIERUNG

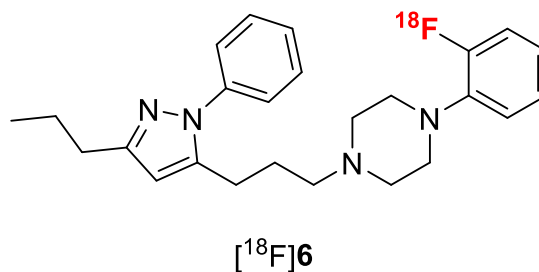
Alkohol unterstützte Cu(II)-vermittelte Radiofluorierung:^[1]



i) elution of $^{18}\text{F}^-$
with Et_4NHCO_3
 $n\text{-BuOH}$
ii) $[\text{Cu}(\text{OTf})_2(\text{py})_4]$
DMA, 110 °C, 10 min



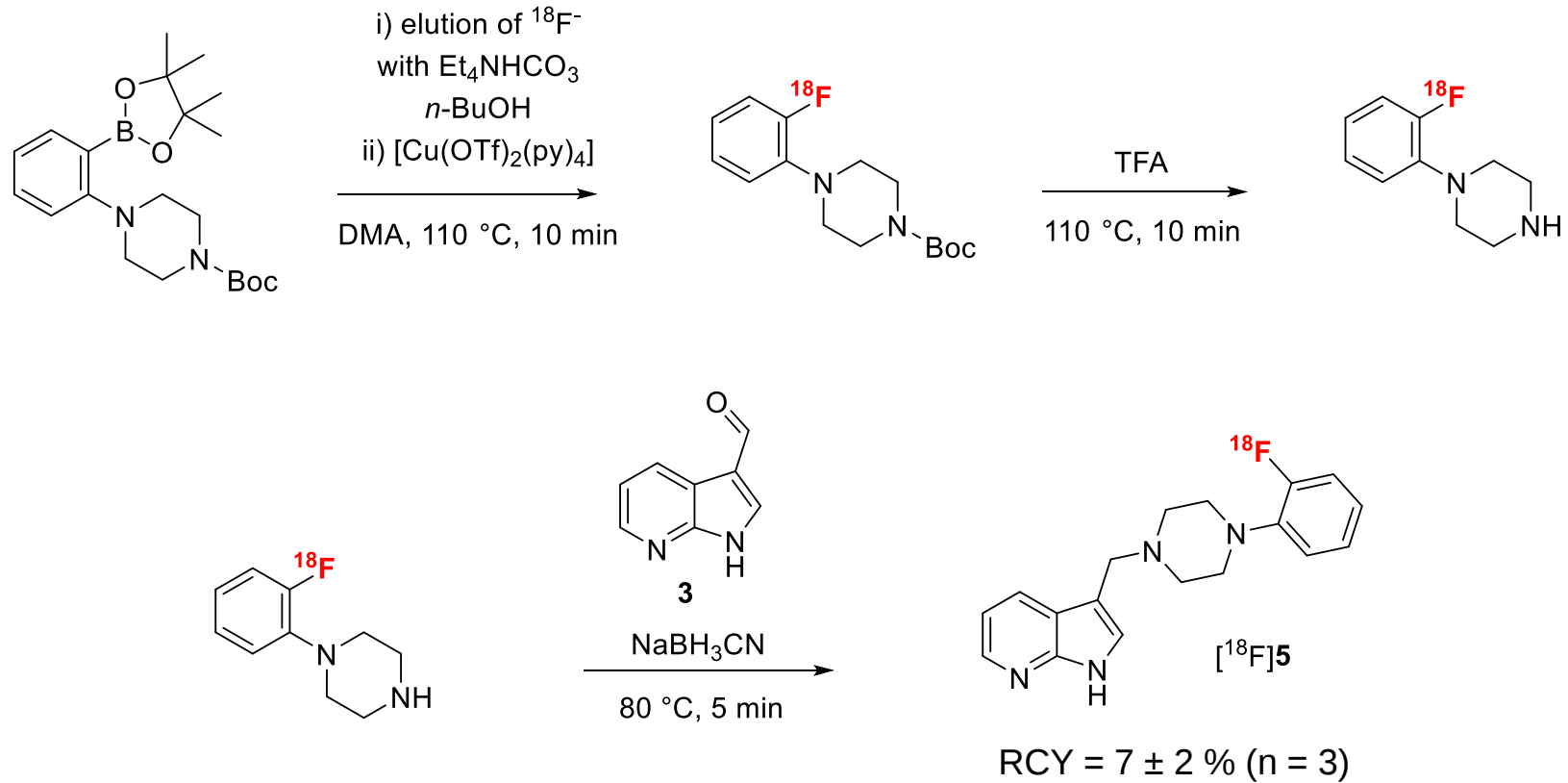
i) elution of $^{18}\text{F}^-$
with Et_4NHCO_3
 $n\text{-BuOH}$
ii) $[\text{Cu}(\text{OTf})_2(\text{py})_4]$
DMA, 110 °C, 10 min



- Anspruchsvolle Vorläufersynthesen.
- *ortho*-Borylierung besonders problematisch
- ^{18}F -Markierungsversuche bisher erfolglos

RADIOFLUORIERUNG

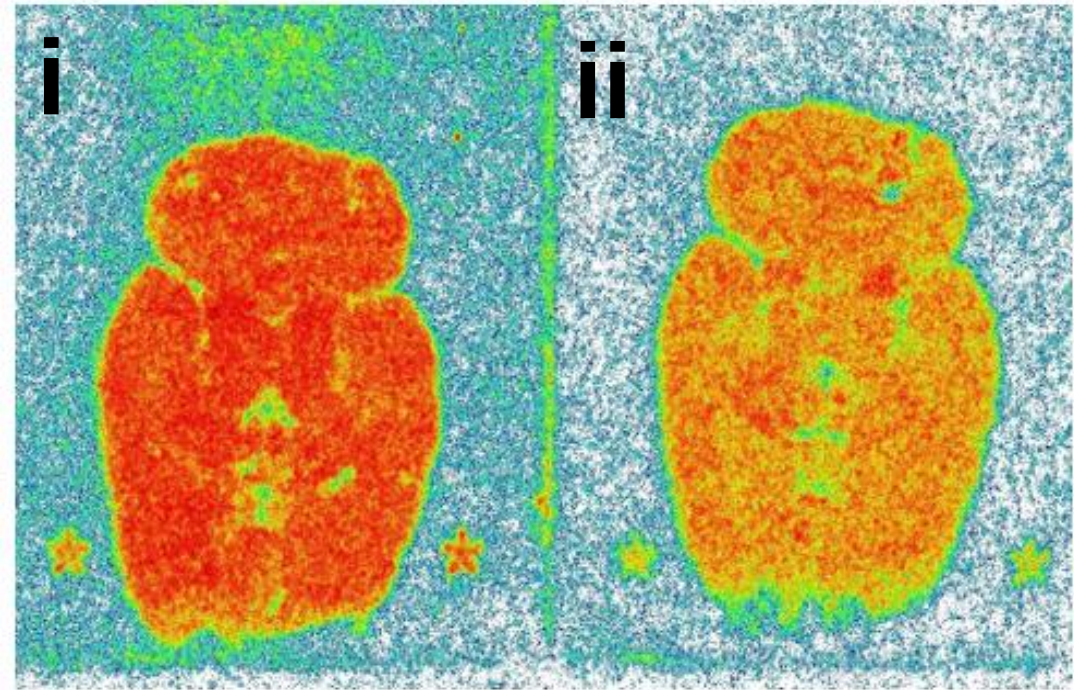
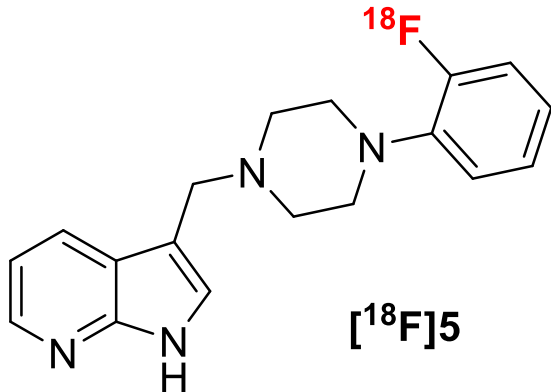
Alternative Route



IN VITRO AUTORADIOGRAPHIE

Autoradiographie mit [^{18}F]5 (2,5 kBq/mL) an horizontalen Rattenhirnschnitten:

- (i) Totalbindung
- (ii) geblockt mit “kaltem” Standard 5 (5 μM) um nicht-spezifische Bindung darzustellen

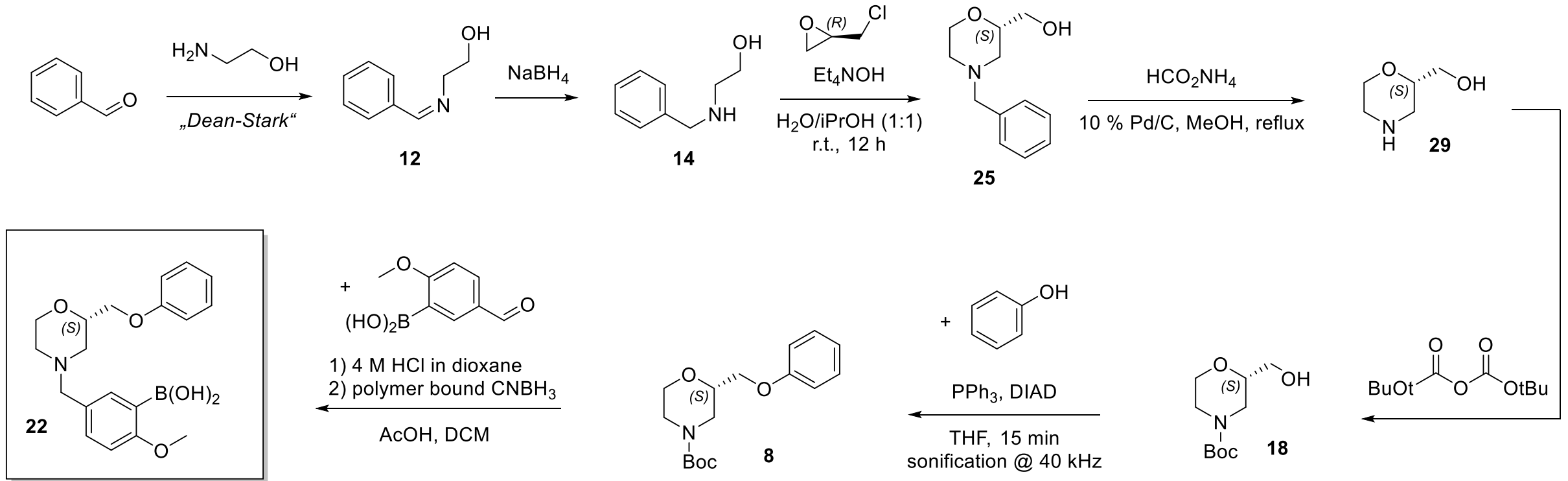


Hoher Anteil an nicht-spezifischer Bindung

- Geringe spezifische Aktivität bei manueller Synthese
- Evtl. zu lipophil?

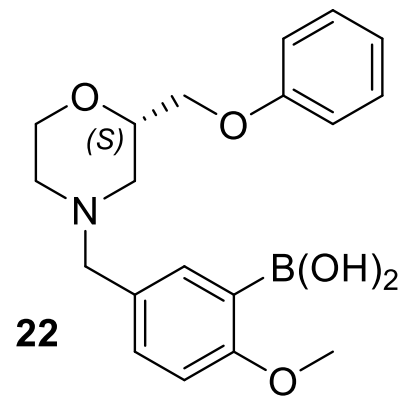
D₄-RADIOLIGANDEN

Vorläufersynthese



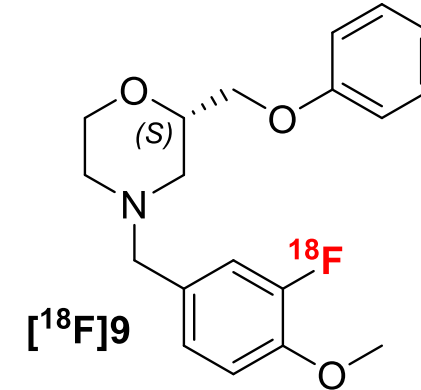
RADIOFLUORIERUNG

Radiosynthese von [^{18}F]9



i) elution of $^{18}\text{F}^-$
with Et_4NHCO_3
 $n\text{-BuOH}$
ii) $[\text{Cu}(\text{OTf})_2(\text{py})_4]$

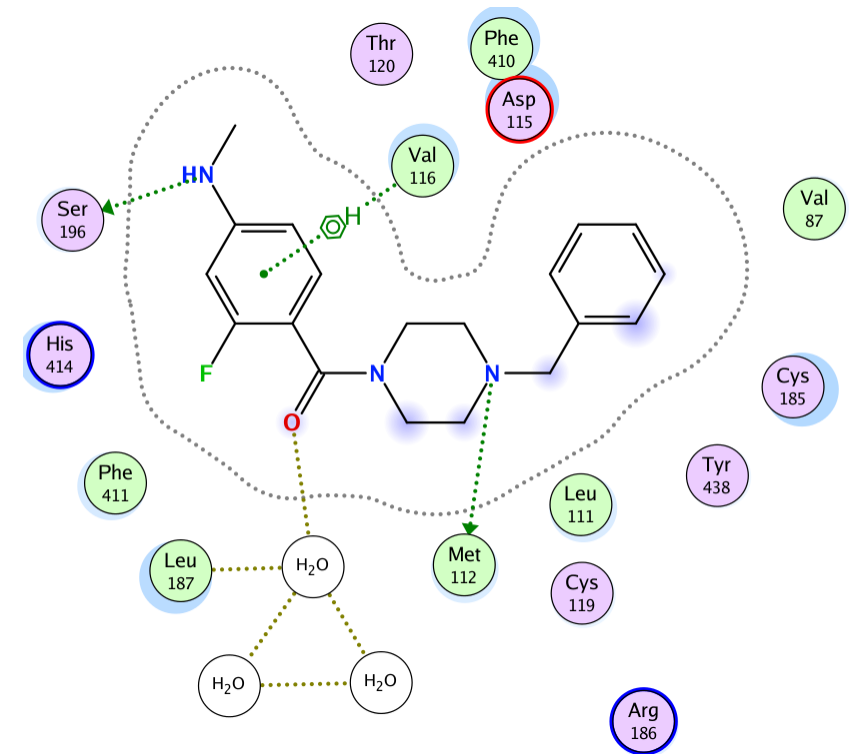
DMA, 110 °C, 10 min



RCY = 66 ± 7 % (n = 4)

ZUSAMMENFASSUNG & AUSBLICK

- ✓ Radiosynthese der neuen ^{18}F -markierten D_4 -Rezeptorliganden $[^{18}\text{F}]\mathbf{5}$ & $[^{18}\text{F}]\mathbf{9}$
- ✓ Erste Resultate durch *in vitro* Autoradiographien
- Automatisierung der Synthesen der Verbindungen $[^{18}\text{F}]\mathbf{5}$ & $[^{18}\text{F}]\mathbf{9}$ für Autoradiographien mit hoher spezifischer Aktivität
- Strukturoptimierungen mit Hilfe von *in silico* Modellen basierend auf der Kristallstruktur des D_4 -Rezeptors^[1]



ACKNOWLEDGMENTS

Prof. Dr. Bernd Neumaier, Prof. Dr. Johannes Ermert, PD Dr. Boris Zlatopolskiy

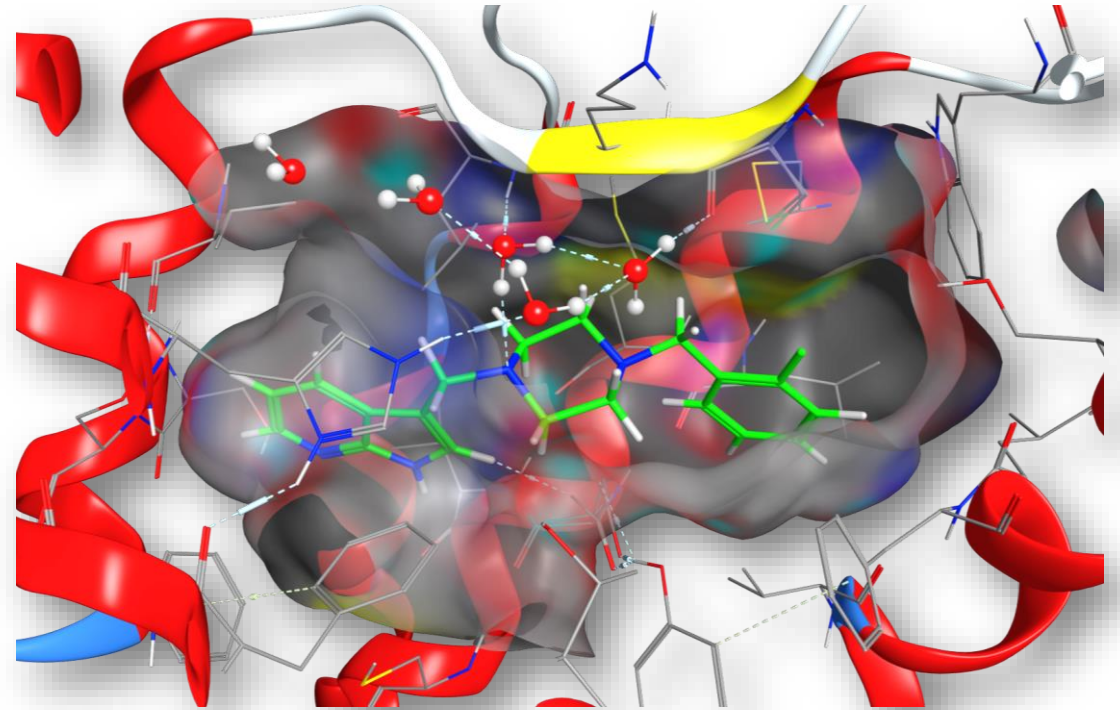
Dr. Dirk Bier, Dr. Marcus Holschbach, Dr. Philipp Krapf, Dr. Johannes Zischler

Drug Design Lab Rossetti: Jonas Goßen, Michael Margreiter, Prof. Dr. Giulia Rossetti

IN SILICO INHIBITOR DESIGN*

Rational Inhibitor Design Based on Protein Databank Structure 5WIV

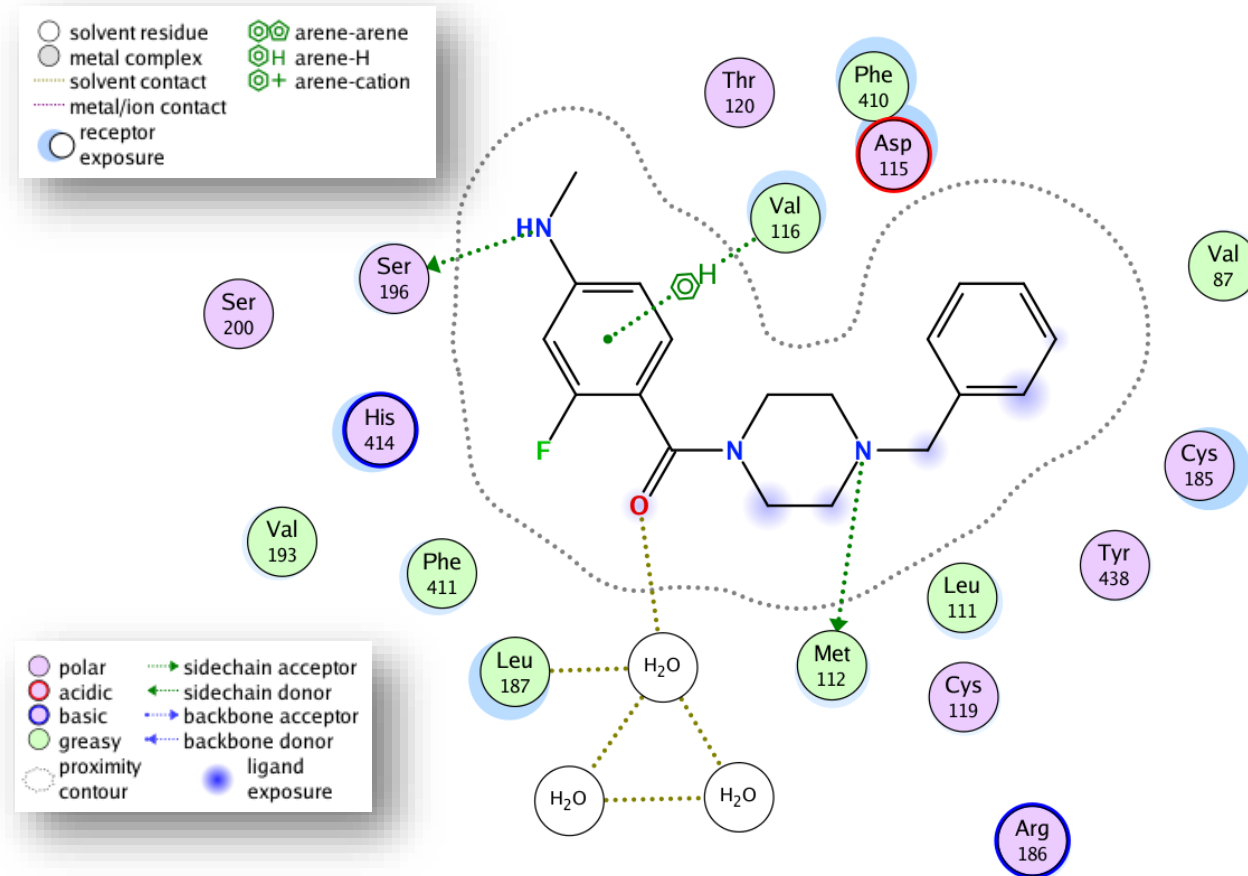
- Structural information on available co-crystallized inhibitors can be leveraged
- Plausible binding hypotheses are generated based on chemical similarity and binding site complementarity
- Prioritize which ligand modifications can increase enthalpic and entropic contributions to binding free energy with molecular dynamics simulations (in progress)



Proposed binding pose of ligands with no cocrystallized structure based on a docking study using the structure 5WIV

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Rational Inhibitor Design Based on Protein Databank Structure 5WIV

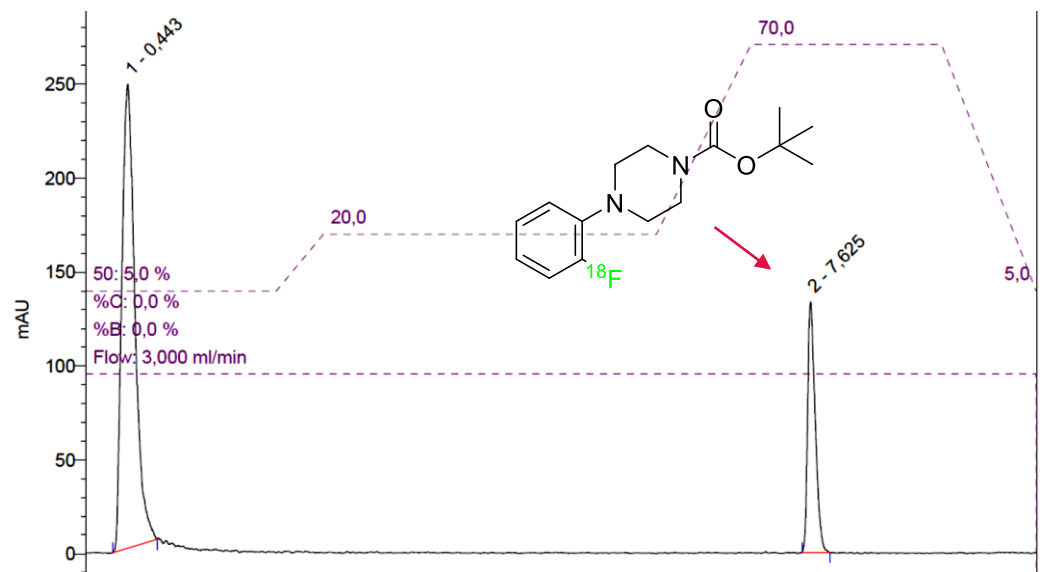


Protein-ligand interaction diagram of a docked ligand

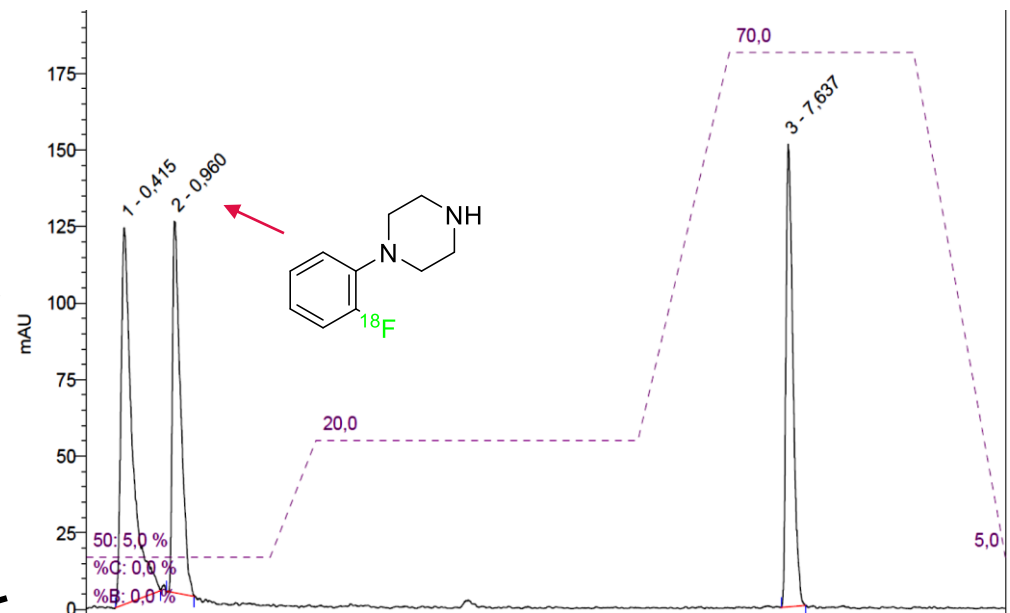
Docking the structure on the left into the known binding site (MOE 2019.2, rigid docking) and retaining crystallographic waters

➤ yielding 5 poses (best one in blue row):

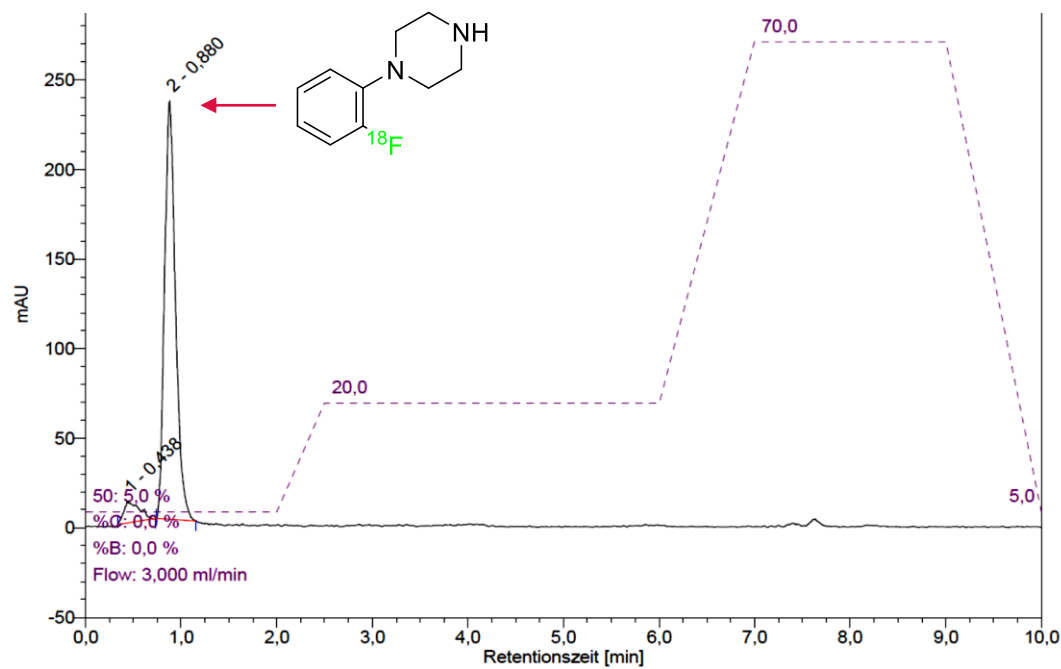
F9	M112	D115	V116	S196	F410	H ₂ O	H ₂ O
1							



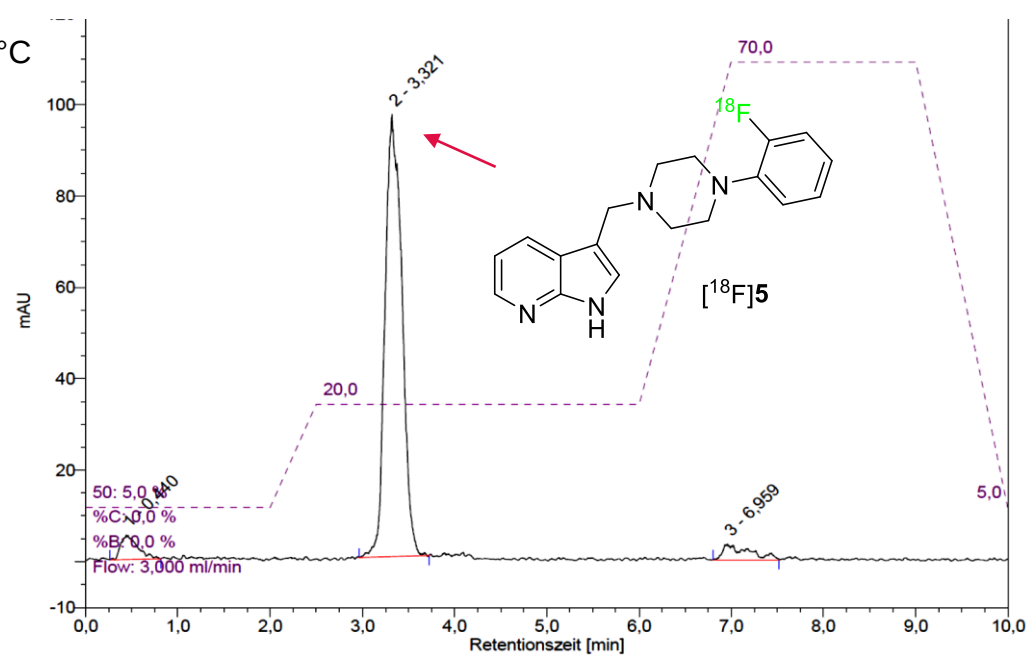
TFA
10 min 60 °C



TFA
10 min 110 °C

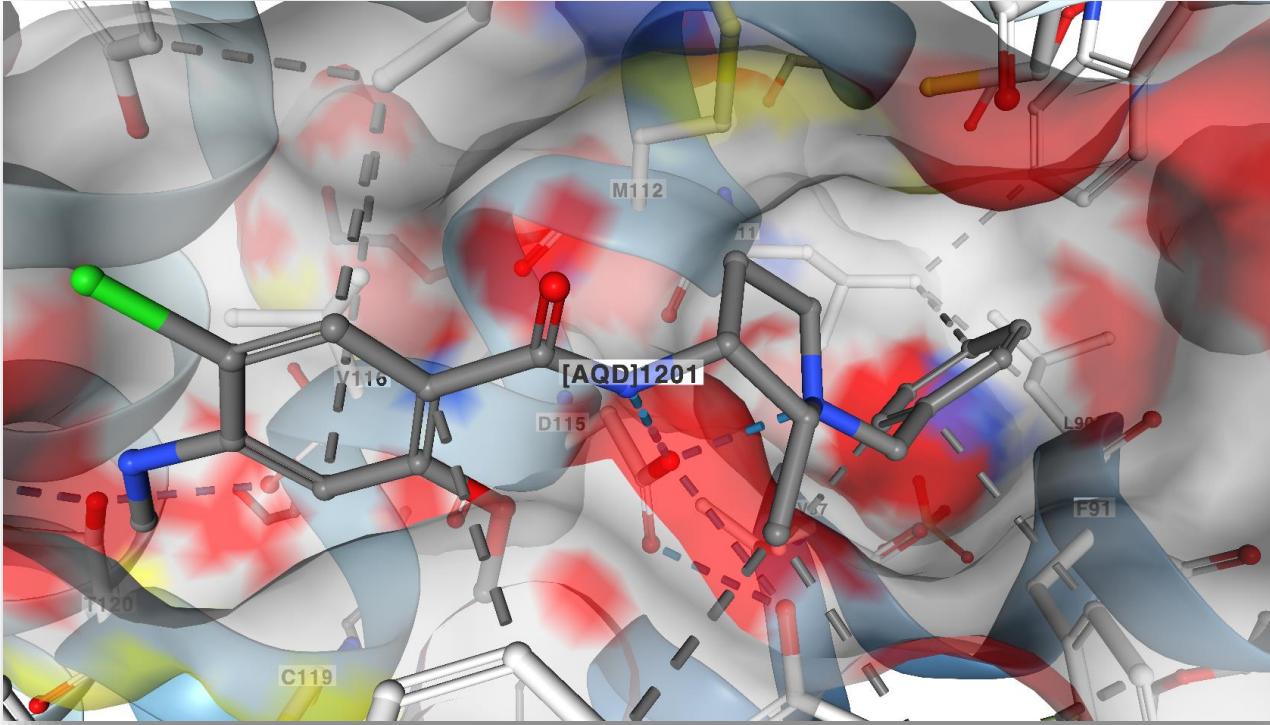


NaCNBH₃
5 min 80 °C

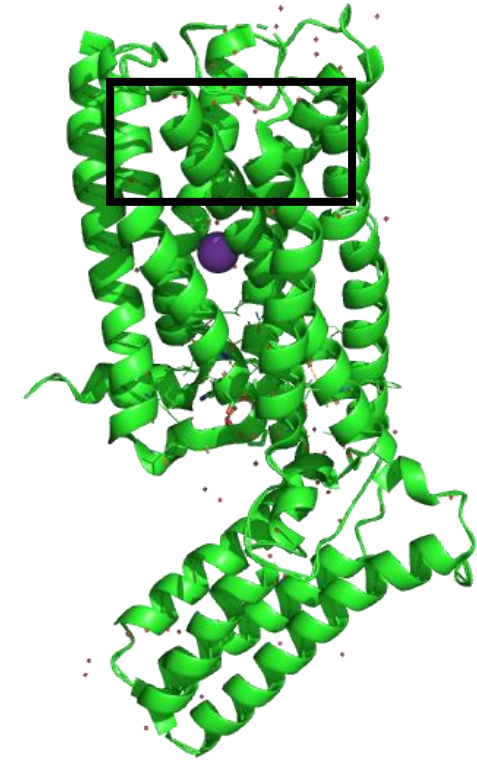


IN SILICO INHIBITOR DESIGN

Rational Inhibitor Design Based on Protein Databank Structure 5WIV



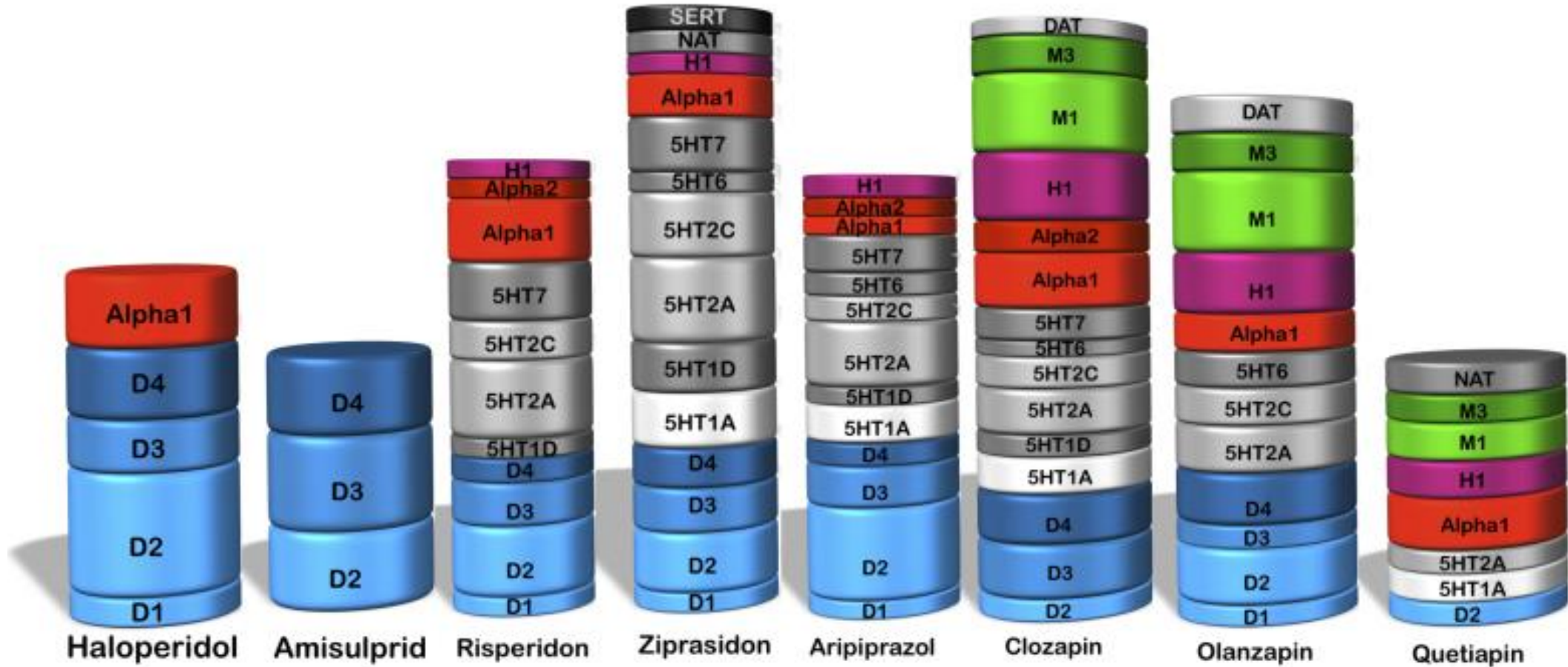
Structure of the sodium-bound human D4 Dopamine receptor in complex with Nemonapride (AQD). PDB Code: 5WIV



Structural information on available co-crystallized inhibitors can be leveraged

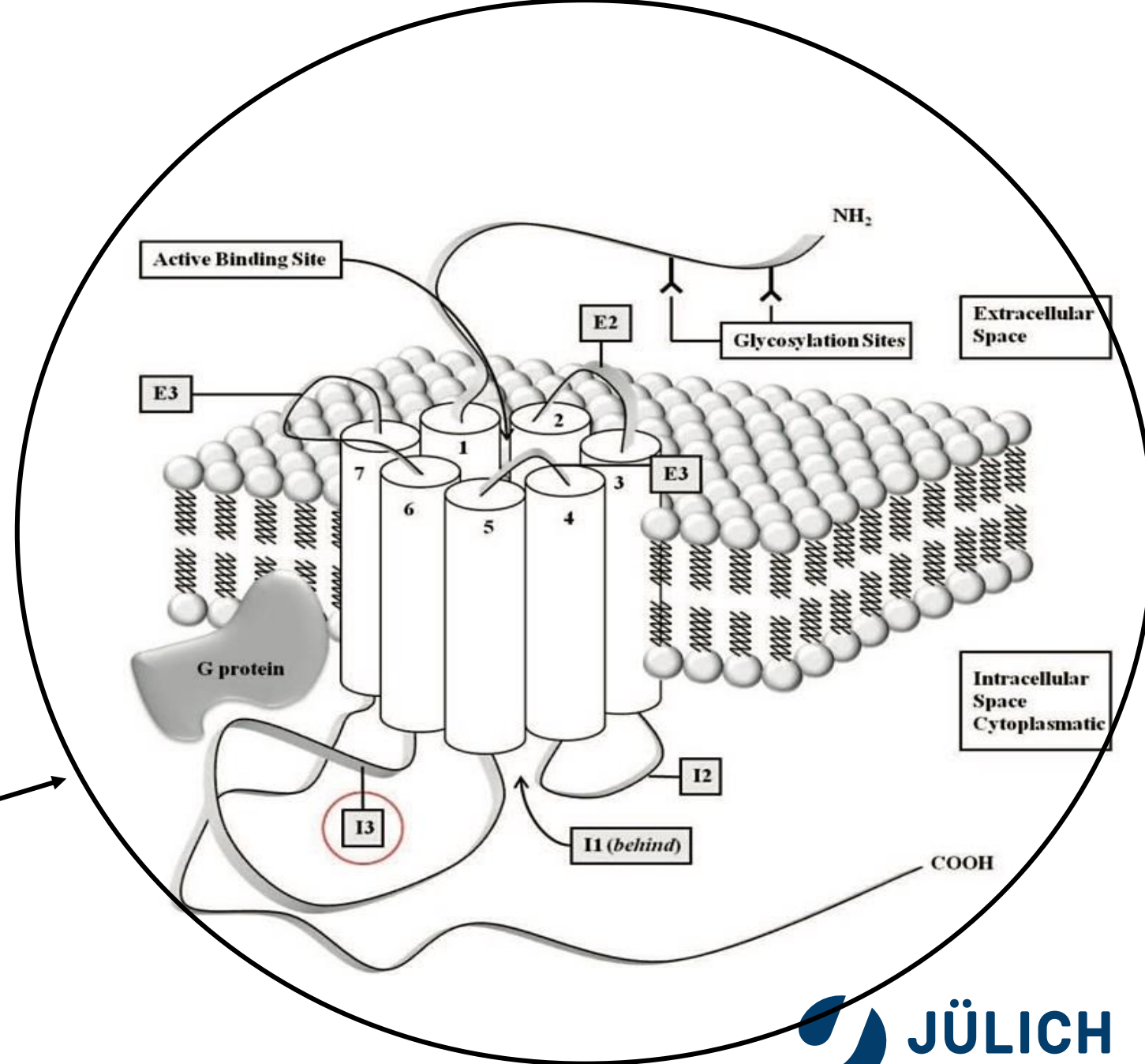
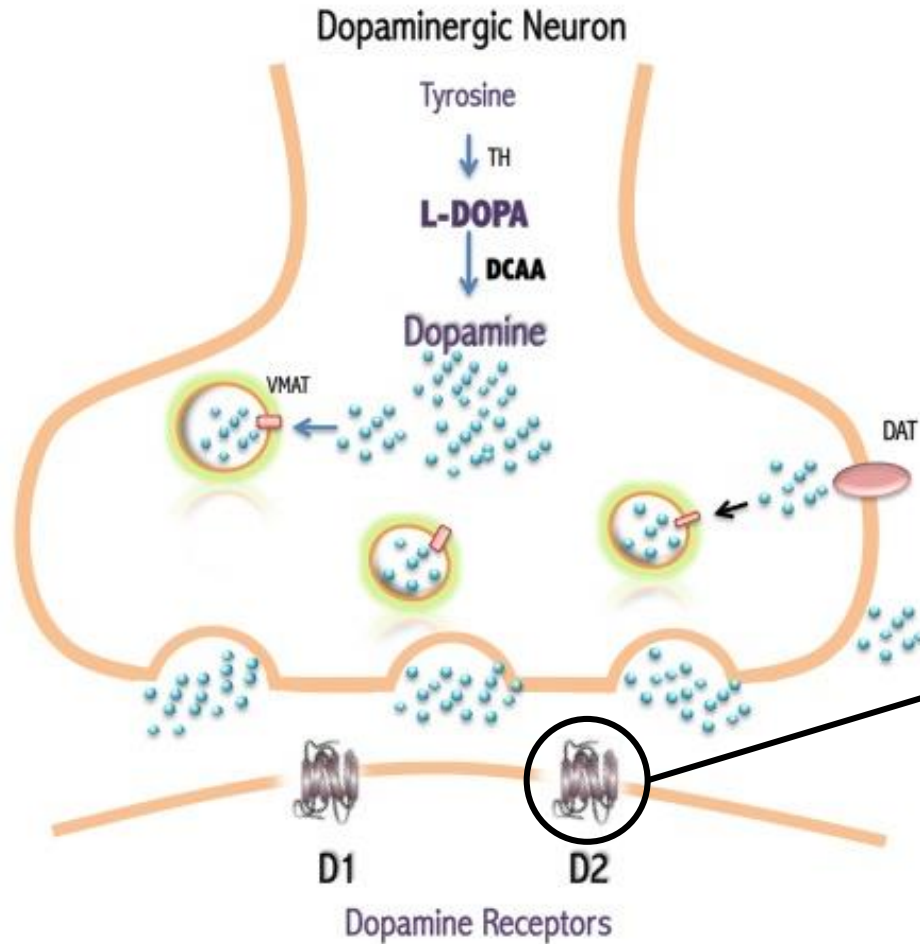
RECEPTORS ADDRESSED BY NEUROLEPTICS

„Rich in Rharmacology“



DOPAMINERGIC SYSTEM

Dopamine



DOPAMINERGIC SYSTEM

Dopamine

