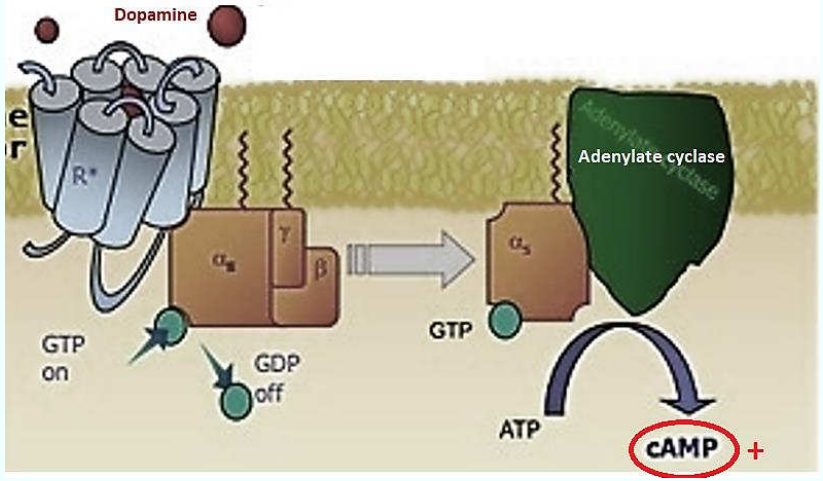
^{18}F-MARKIERTE PHENYLPIPERAZINE ALS NEUE STRUKTURMOTIVE FÜR SPEZIFISCHE DOPAMIN D_4-REZEPTORLIGANDEN

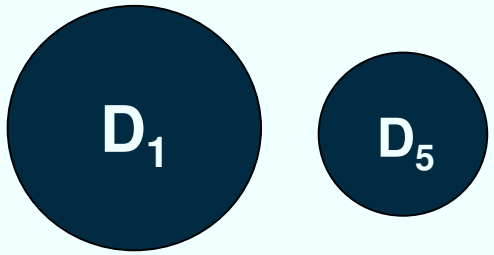
05.04.2019 | Michael Willmann

Forschungszentrum Jülich
Institut für Neurowissenschaften und Medizin (INM-5)

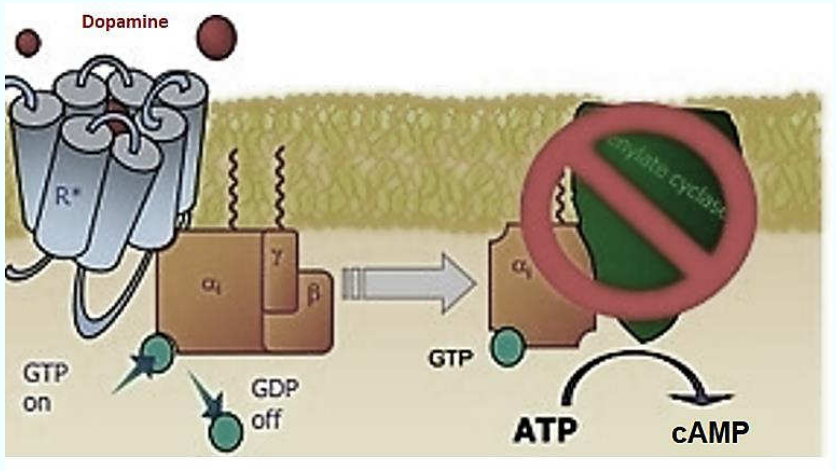
DOPAMIN-REZEPTOR-SUBTYPEN

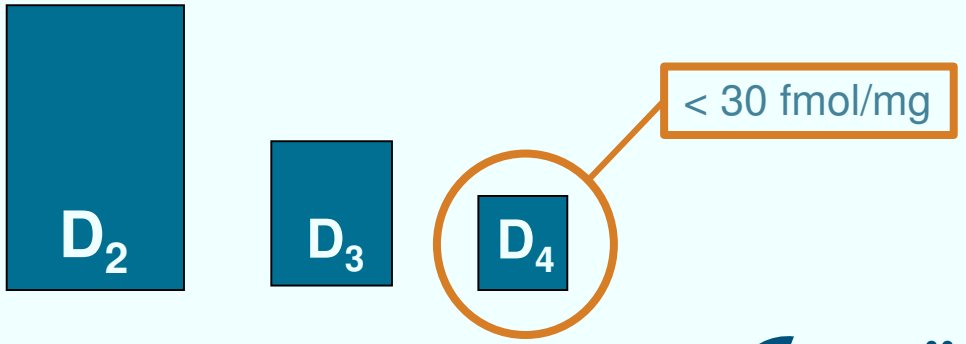
D_1–Familie:





D_2–Familie:





WARUM DER D_4-REZEPTOR?

„Terra incognita“

Clozapin: Erstes atypisches Neuroleptikum (1972):

- D_4-selektiv
- Kein EPS* als Nebenwirkung

*Extrapyramydalmotorisches Syndrom

Aktuelles Interesse am D_4-Rezeptor (ab 1998):[1]

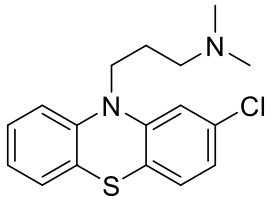
ADHS[1]

Sucht (Kokain, Amphetamine)[2]

Libido[3]

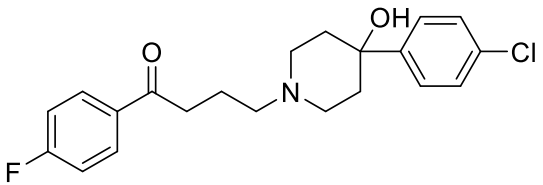
Parkinson[1]

Tumortherapie (Glioblastoma)[4]



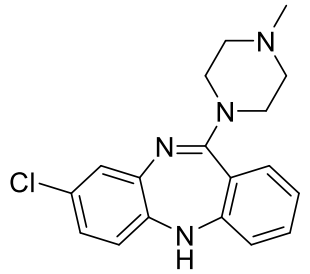
Chlorpromazin

$K_i(D_1)$ 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
- Tumortherapie (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,

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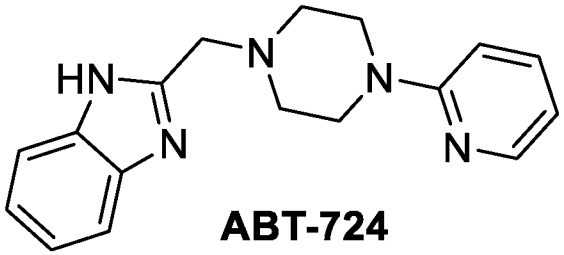
2004

Activation of dopamine D_4 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



ABT-724

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2012

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. Koprich, 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

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- Sucht (Kokain, Amphetamine)

2016

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. Selvadurai[1], Xiaoyang Lan[1,3], Lilian Lee[1], Michelle Kushida[1], Veronique Voisin[5], Heather Whetstone[1], Milly So[1], Tzvi Aviv[1], Nicole Park[1,3], Xueming Zhu[1], ChangJiang Xu[5], Renee Head[1], Katherine J. Rowland[1], Mark Bernstein[6], Ian D. Clarke[1,7], Gary Bader[3,5], Lea Harrington[8], John H. Brumell[3,9], Mike Tyers[8], and Peter B. Dirks[1,2,3,4,*]

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- ADHS
- Libido
- Parkinson
- Tumortherapie (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 WANG[1], WENWEN SHEN[2], LONGHUI LI[2], XIAOHU XIE[2], HAOCHANG HU[1], LEI XU[1], WENHUA ZHOU[2] and SHIWEI DUAN[1]

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

Received May 4, 2016; Accepted April 10, 2017

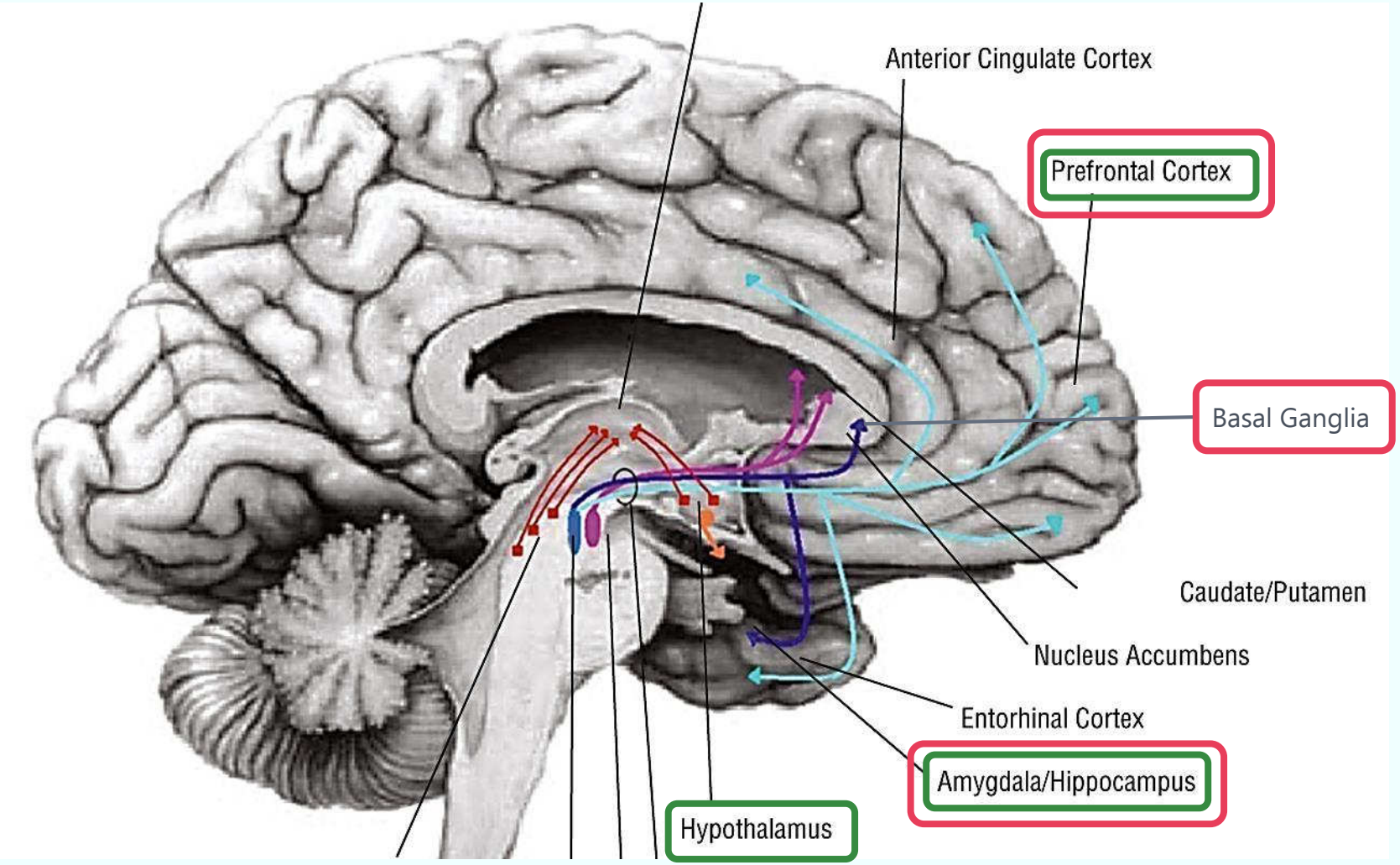
D_4-Rezeptorligand als „Goldstandard“ essentiell für tiefergehendes Verständnis

DOPAMIN D_4-REZEPTOR

Lokalisation im Gehirn

in situ Hybridisierung

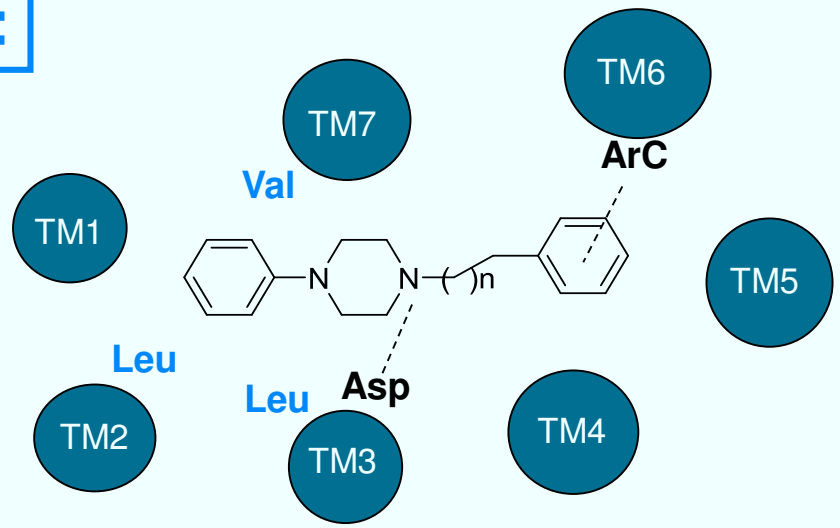
Substraktive Methoden



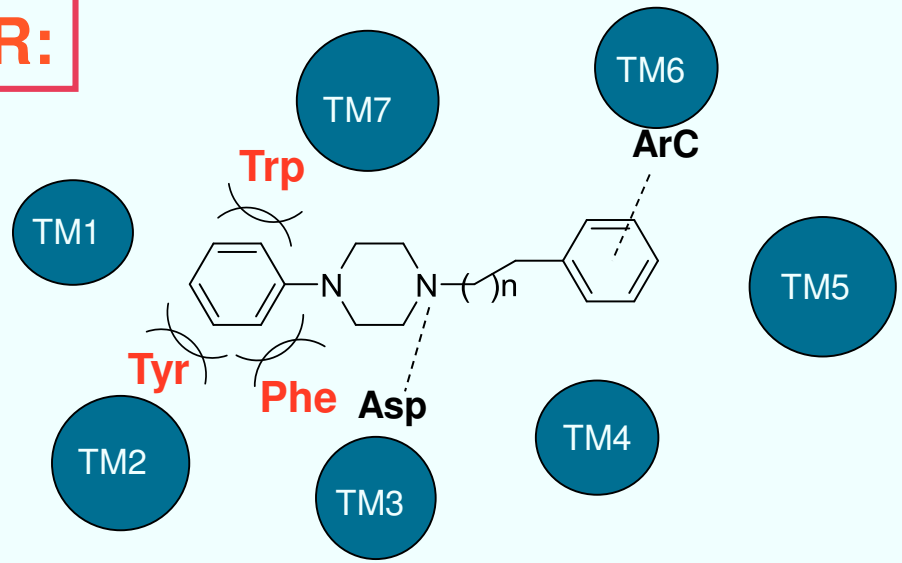
„STRUCTURE AFFINITY RELATIONSHIPS“ (SAFIR)

Worauf beruhen Affinität und Selektivität?

D_4R:



D_2R:



Affinität:

ArC	**Ar**omatic **C**omplex
Asp	-CH_2COOH
*TM 1-7:	**Trans**membran Helices

Selektivität:

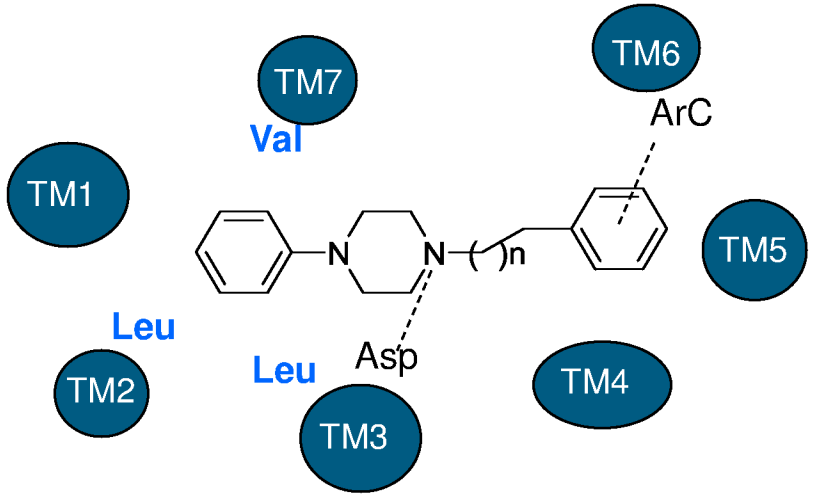
D_2R:	Trp	-$CH_2C_8H_6N$	D_4R:	Leu	-$CH_2CH_2(CH_3)_2$
	Phe	-$CH_2C_6H_5$		Val	-$CH_2(CH3)_2$
	Tyr	-$CH_2C_6H_4OH$			

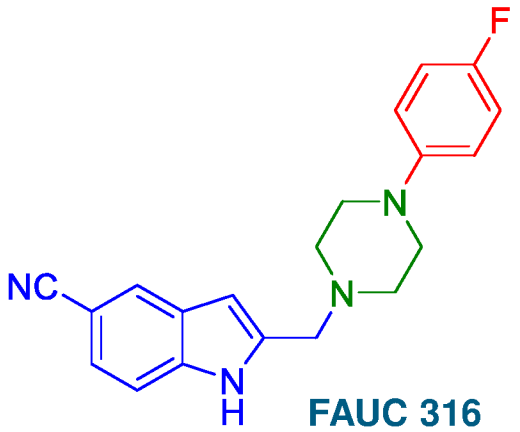
JÜLICH Forschungszentrum

modified from: Simpson, M. M., et al. (**1999**). *Mol. Pharm.* **56**: 1116-1126.

„STRUCTURE AFFINITY RELATIONSHIPS“ (SAFIR)

Abgeleitete Leitstrukturen





FAUC 316

$K_i(D_2)$ > 19000 nM
$K_i(D_3)$ > 15000 nM
$K_i(D_4)$ = 1 nM

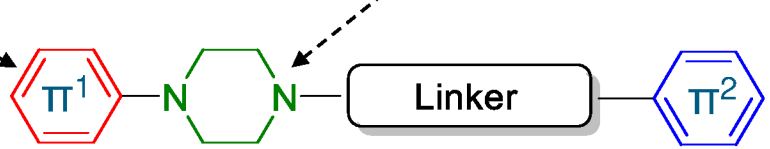
cLogP = 3,54

In vitro Autoradiographien:[1]

- nicht-spezifische Bindung
- zu lipophil

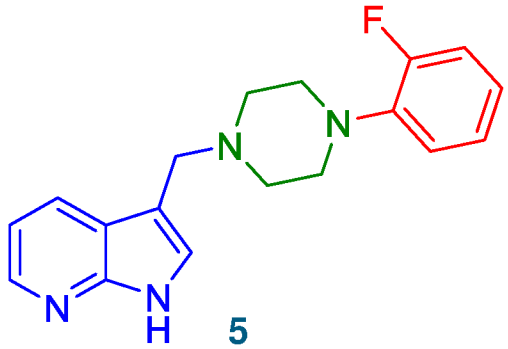
„starres“ Arylamin

Asp (TM3)



Linker:
CH_2: D_4-Selektivität

2. Aryl: erhöht Affinität über π-π Wechselwirkungen zu ArC (TM6)



5

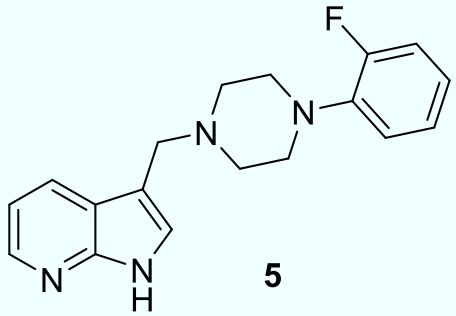
$K_i(D_2)$ > 5000 nM
$K_i(D_3)$ > 2500 nM
$K_i(D_4)$ = 3,11 nM

cLogP = 3,14

- vergleichsweise Hydrophil

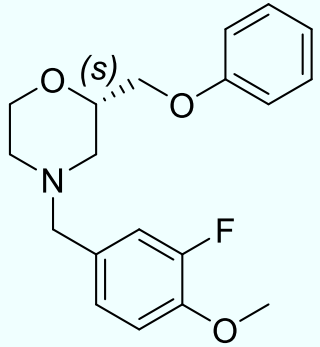
[1] F. Kügler, P. Gmeiner, O. Prante, H. H. Coenen, *J. Med. Chem.* **2011**, *54*, 8343-8352.

AUSGEWÄHLTE D_4-REZEPTORLIGANDEN



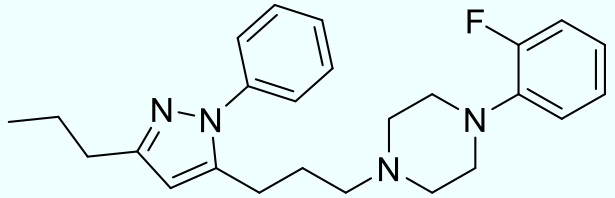
5

$K_i(D_2)$ > 5000 nM
$K_i(D_3)$ 2900 ± 151 nM
$K_i(D_4)$ **3,11** ± 0,85 nM
*c*Log *P* 3,14



9

$K_i(D_4)$ **14,3** nM
*c*Log *P* 3,18



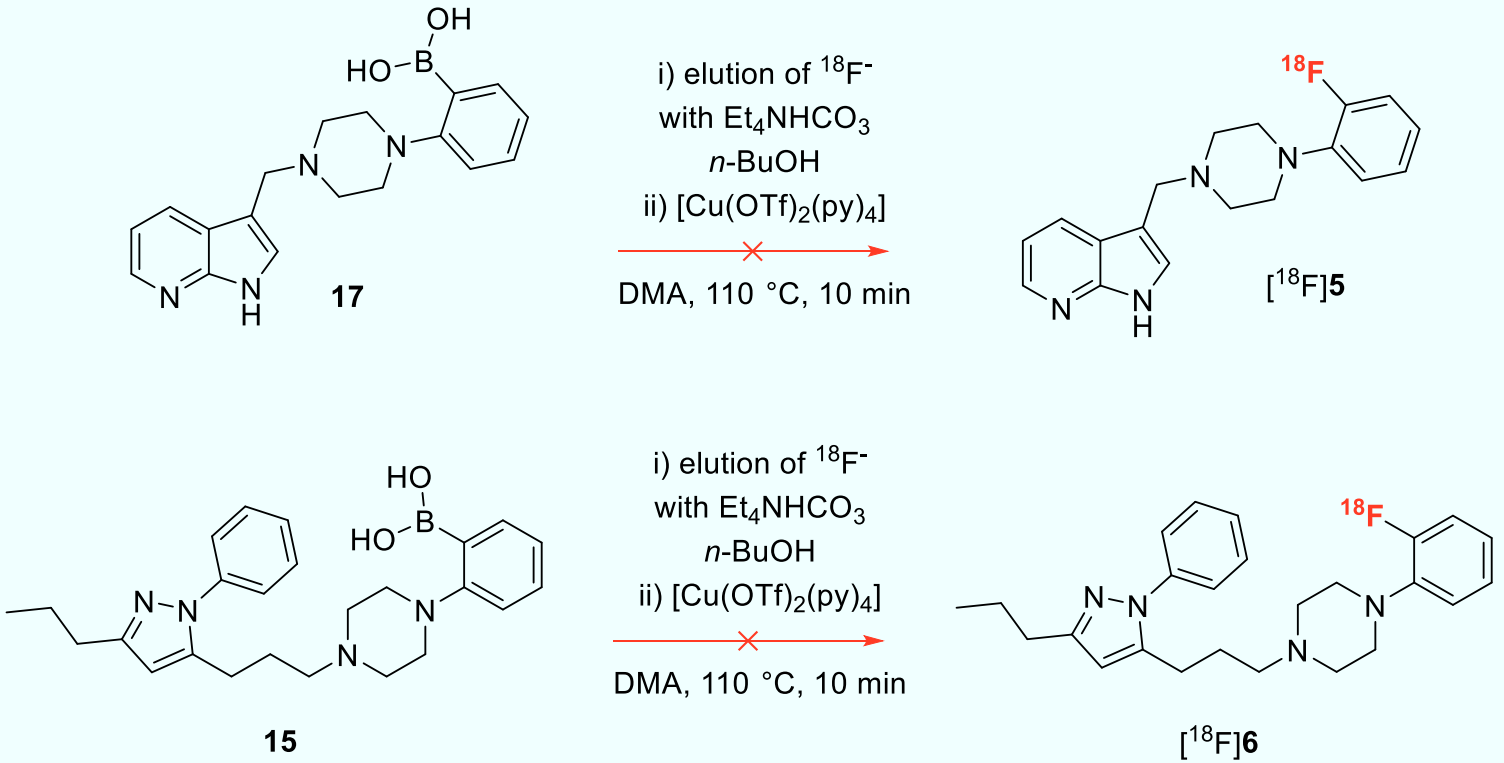
6

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

[1] Oh, S. J., et al. (2004). Bioorg. Med. Chem. **12**(21): 5505-5513.
[2] Kong, J. Y., et al. (2008). Patent: EP212
[3] Witt, J. O., et al. (2016). Bioorg. Med Chem. Lett. **26**(10): 2481-2488.

RADIOFLUORIERUNG

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

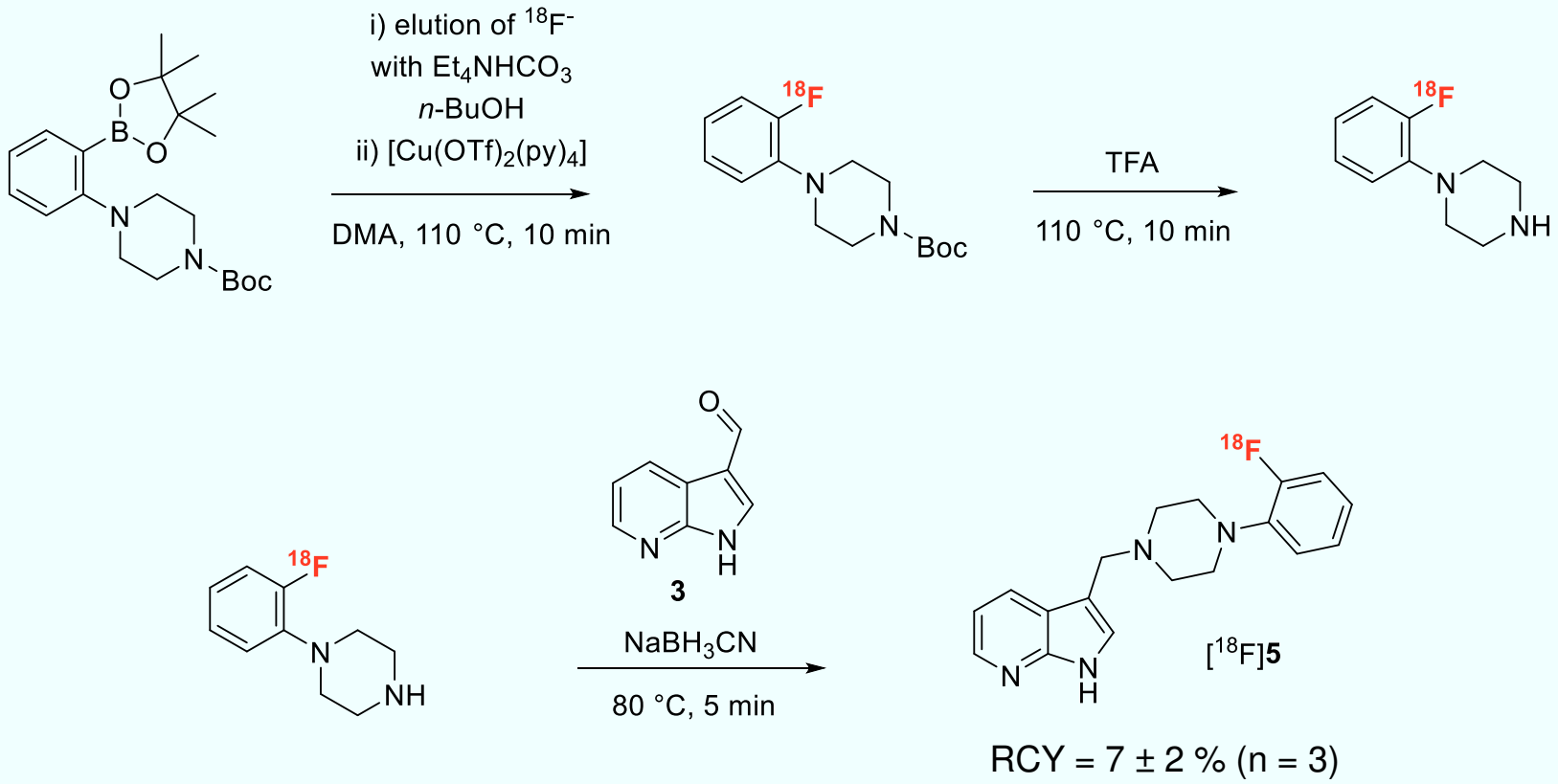


- Anspruchsvolle Vorläufersynthesen.
- *ortho*-Borylierung besonders problematisch
- ^{18}F-Markierungsversuche bisher erfolglos

[1] Zischler, J., et al. (2017). Chemistry – A European Journal **23**(14): 3251-3256.

RADIOFLUORIERUNG

Alternative Route

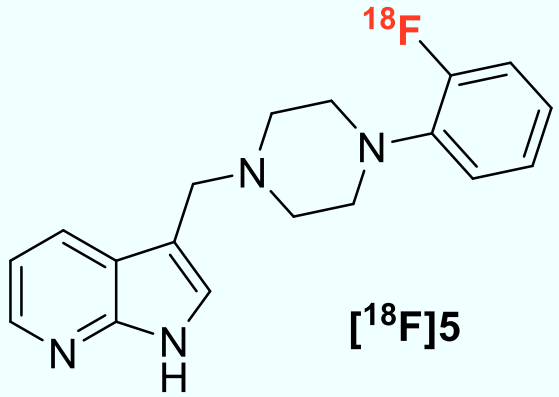


IN VITRO AUTORADIOGRAPHIE

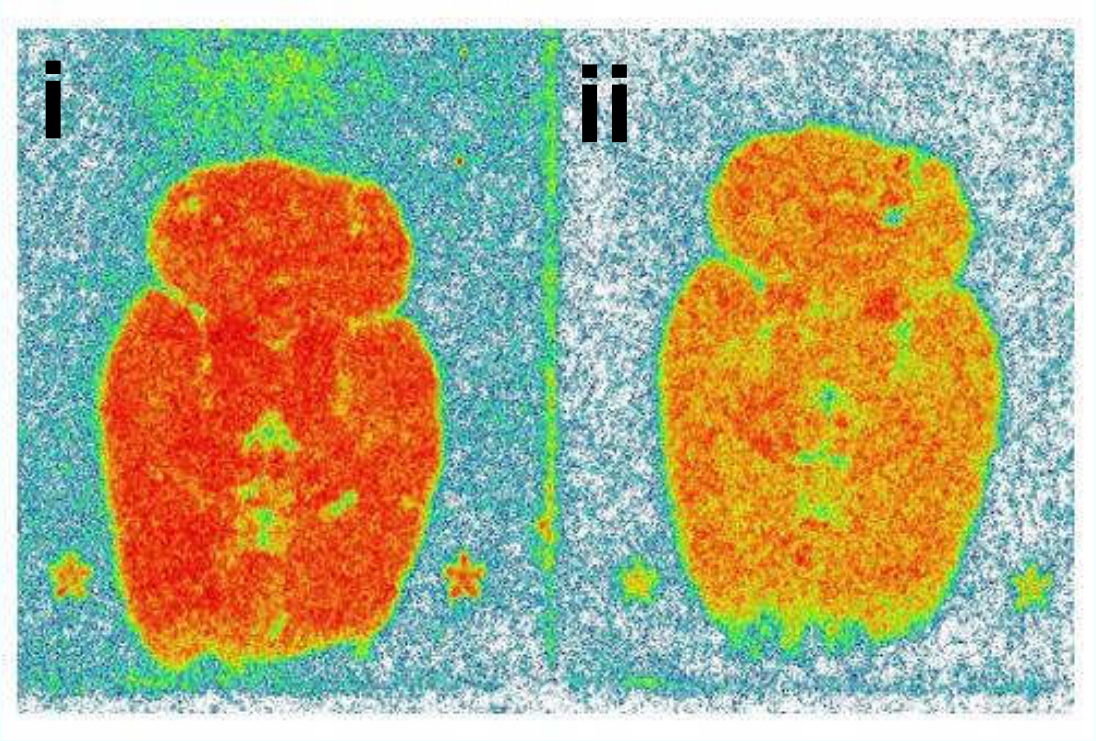
Autoradiographie mit **[^{18}F]5** (2,5 kBq/mL) an horizontalen Rattenhirnschnitten:

(i) Totalbindung

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



[^{18}F]5

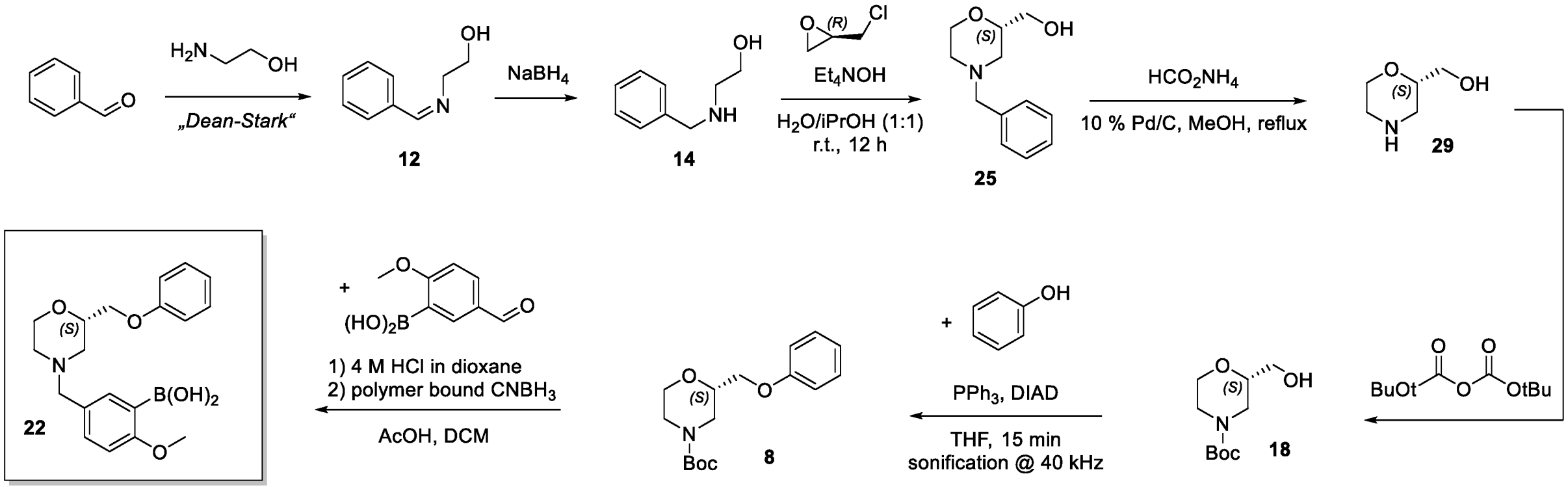


Hoher Anteil an nicht-spezifischer Bindung

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

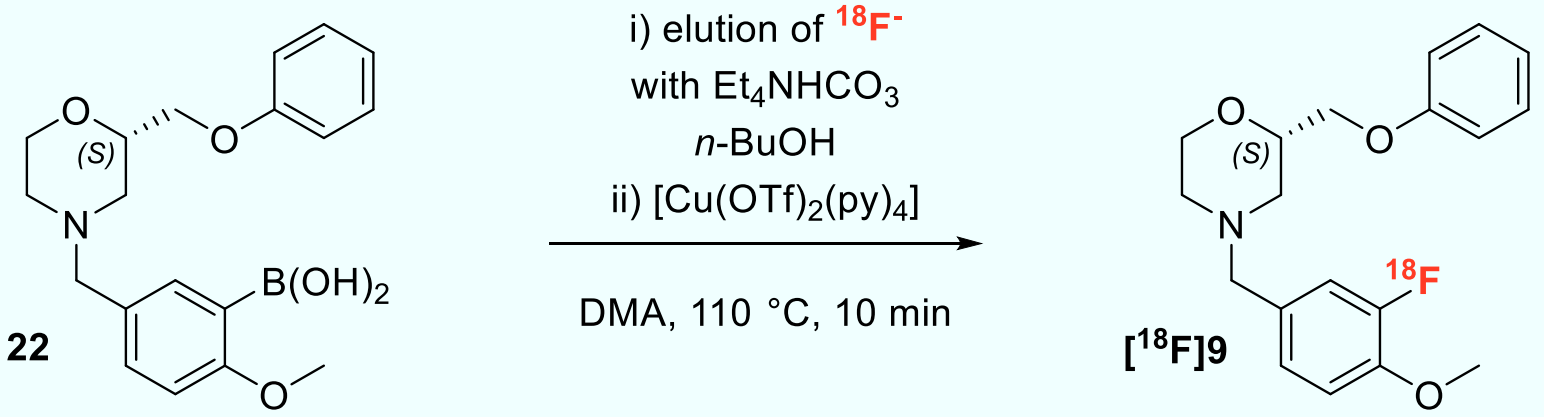
D_4-RADIOLIGANDEN

Vorläufersynthese



RADIOFLUORIERUNG

Radiosynthese von [^{18}F]9



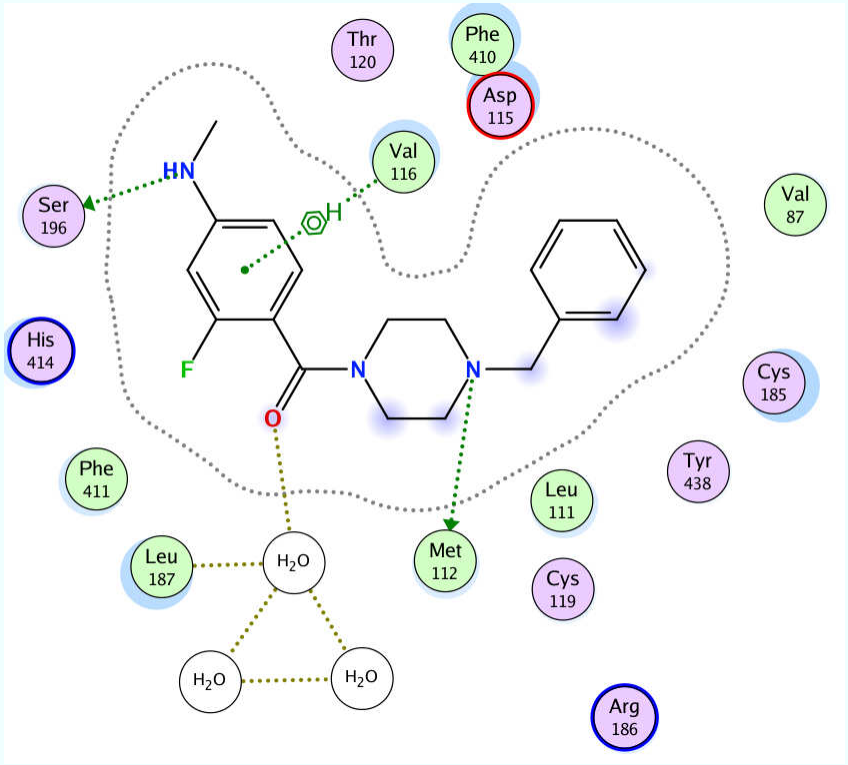
RCY = 66 ± 7 % (n = 4)

[1] Zischler, J., et al. (**2017**). *Chemistry – A European Journal* **23**(14): 3251-3256.

ZUSAMMENFASSUNG & AUSBLICK

- ✓ Radiosynthese der neuen ^{18}F-markierten D_4-Rezeptorliganden [^{18}F]**5** & [^{18}F]**9**
- ✓ Erste Resultate durch *in vitro* Autoradiographien

- ○ Automatisierung der Synthesen der Verbindungen [^{18}F]**5** & [^{18}F]**9** für Autoradiographien mit hoher spezifischer Aktivität
- ○ Strukturoptimierungen mit Hilfe von *in silico* Modellen basierend auf der Kristallstruktur des D_4-Rezeptors[1]



[1] S. Wang, B. L. Roth, *Science* ***2017, 358, 381-386.***

ACKNOWLEDGMENTS

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

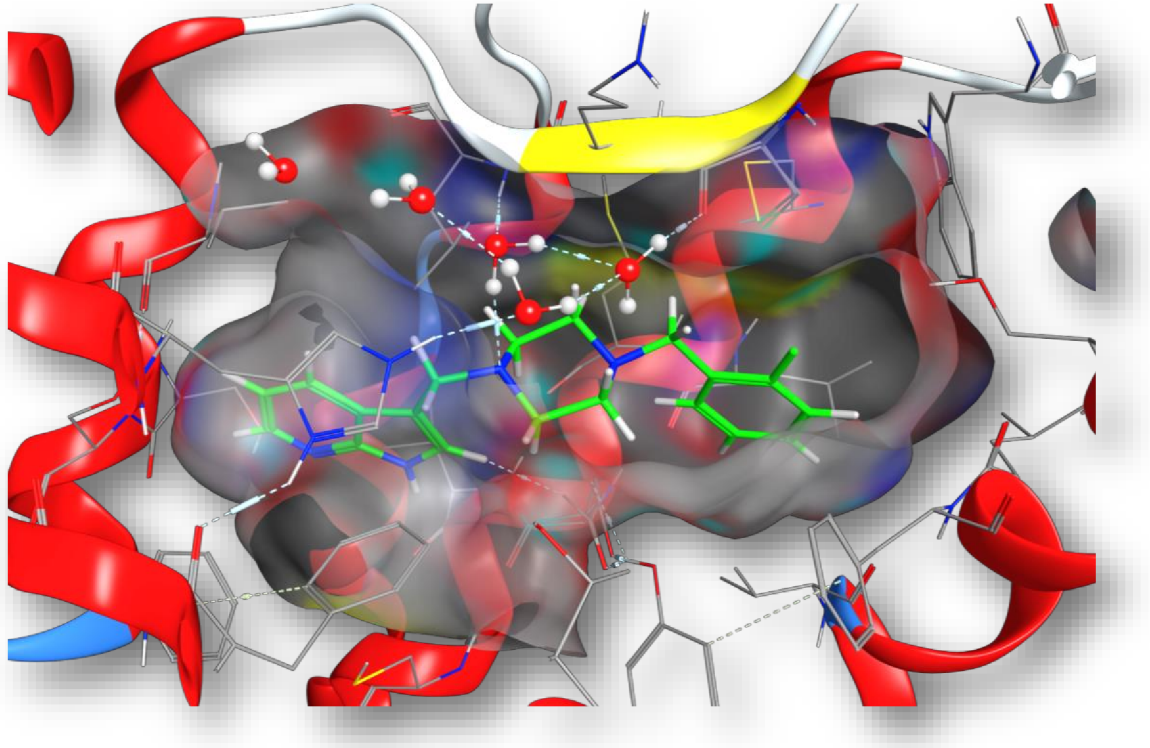
Dr. Dirk Bier, Dr. Marcus Holschbach, Dr. Philipp Krapf, Dr. Johannes Zischler

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-crystalized 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)

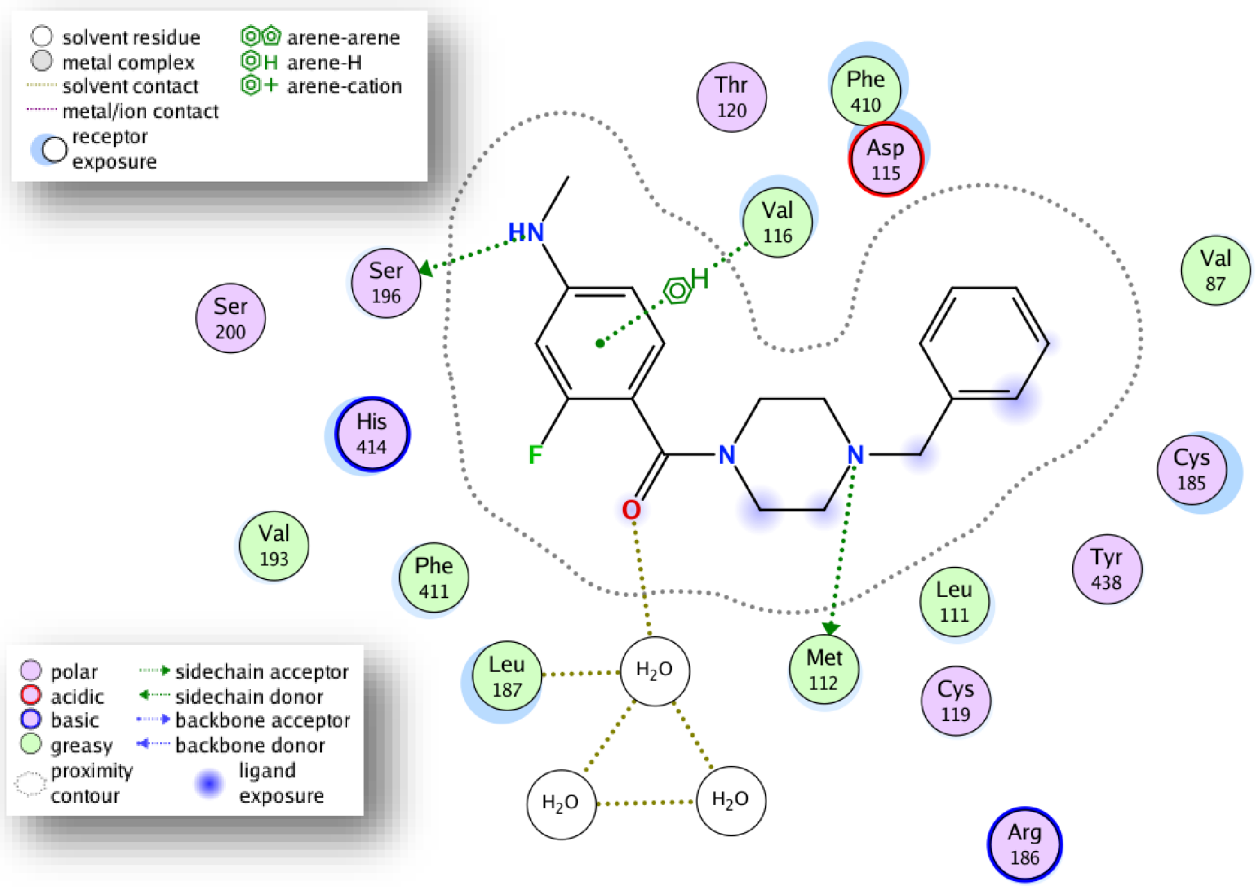


Proposed binding pose of ligands with no cocrystallized struture based on a docking study using the structure 5WIV

**Drug Design Lab Rossetti*: Jonas Goßen, Michael Margreiter, Jun.-Prof. Giulia Rossetti

IN SILICO INHIBITOR DESIGN*

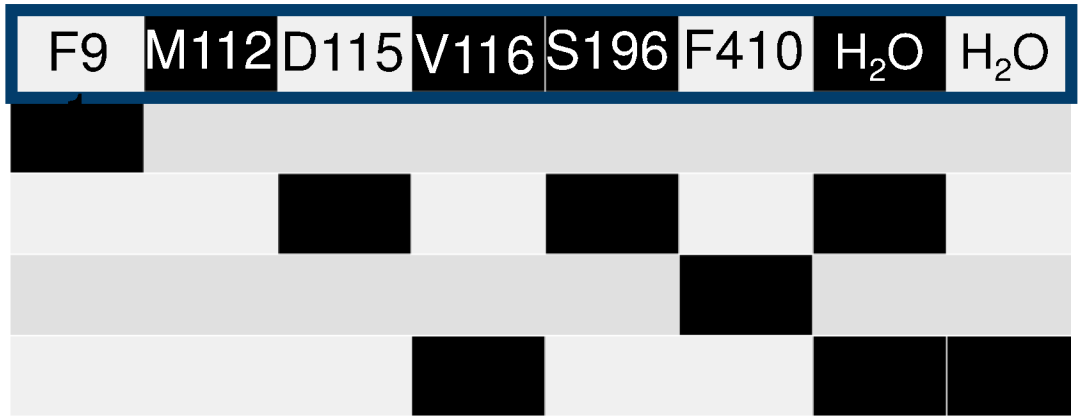
Rational Inhibitor Design Based on Protein Databank Structure 5WIV



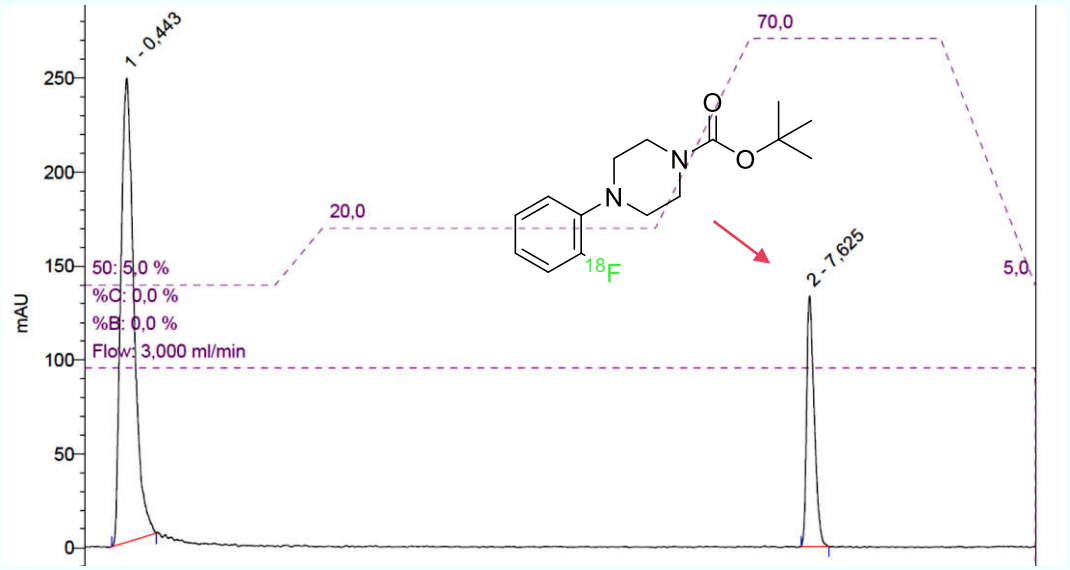
Protein-ligand interaction diagram of a docked ligand

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):

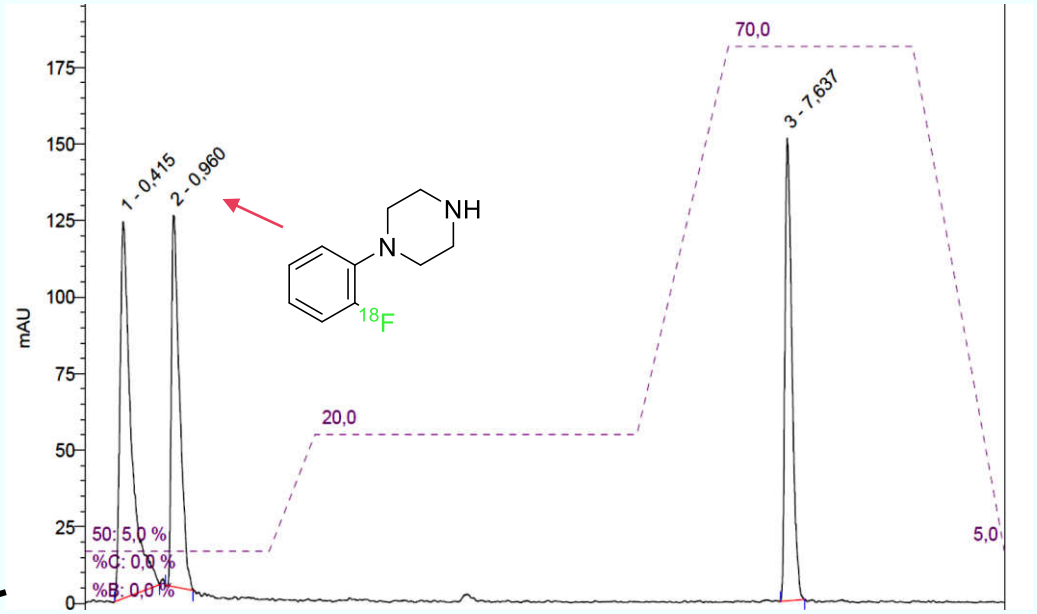


Drug Design Lab Rossetti: Jonas Goßen, Michael Margreiter, Jun.-Prof. Giulia Rossetti



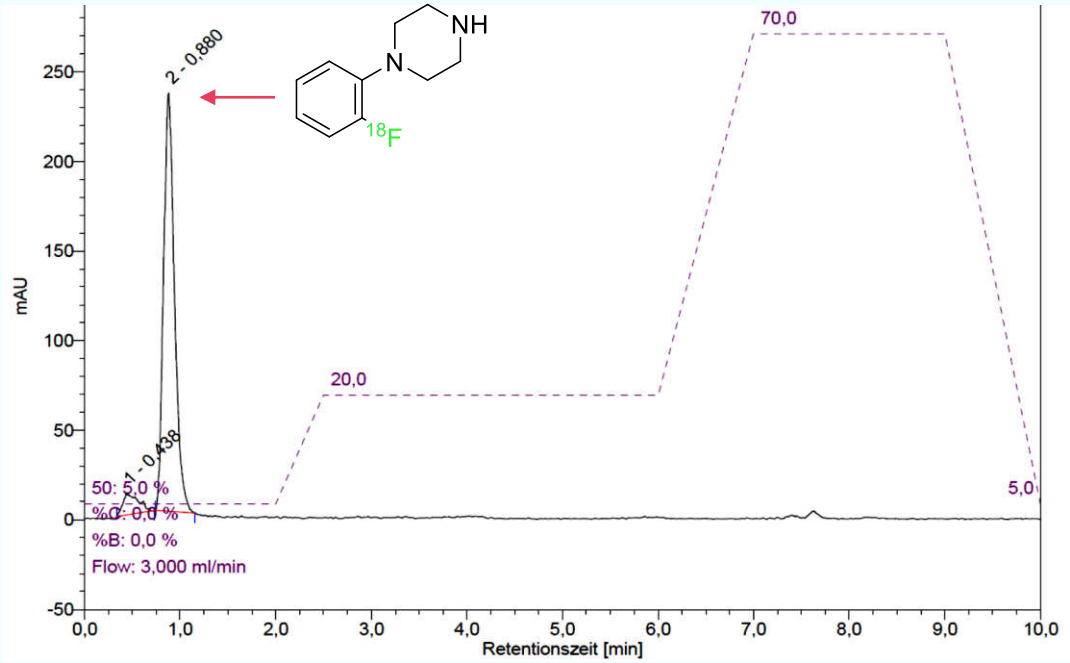
TFA

10 min 60 °C



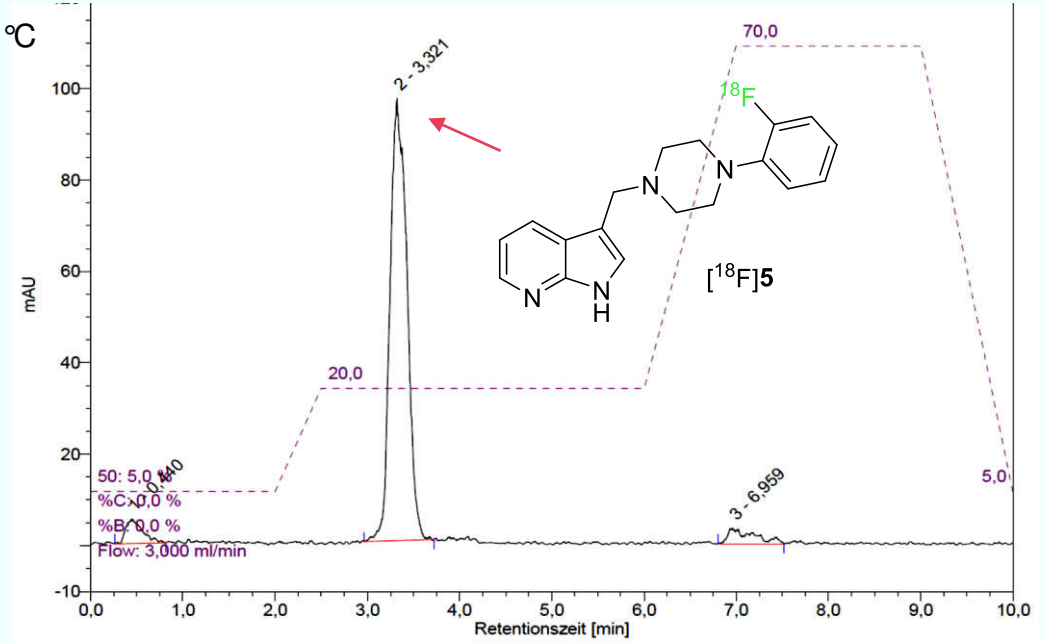
TFA

10 min 110 °C



$NaCNBH_3$

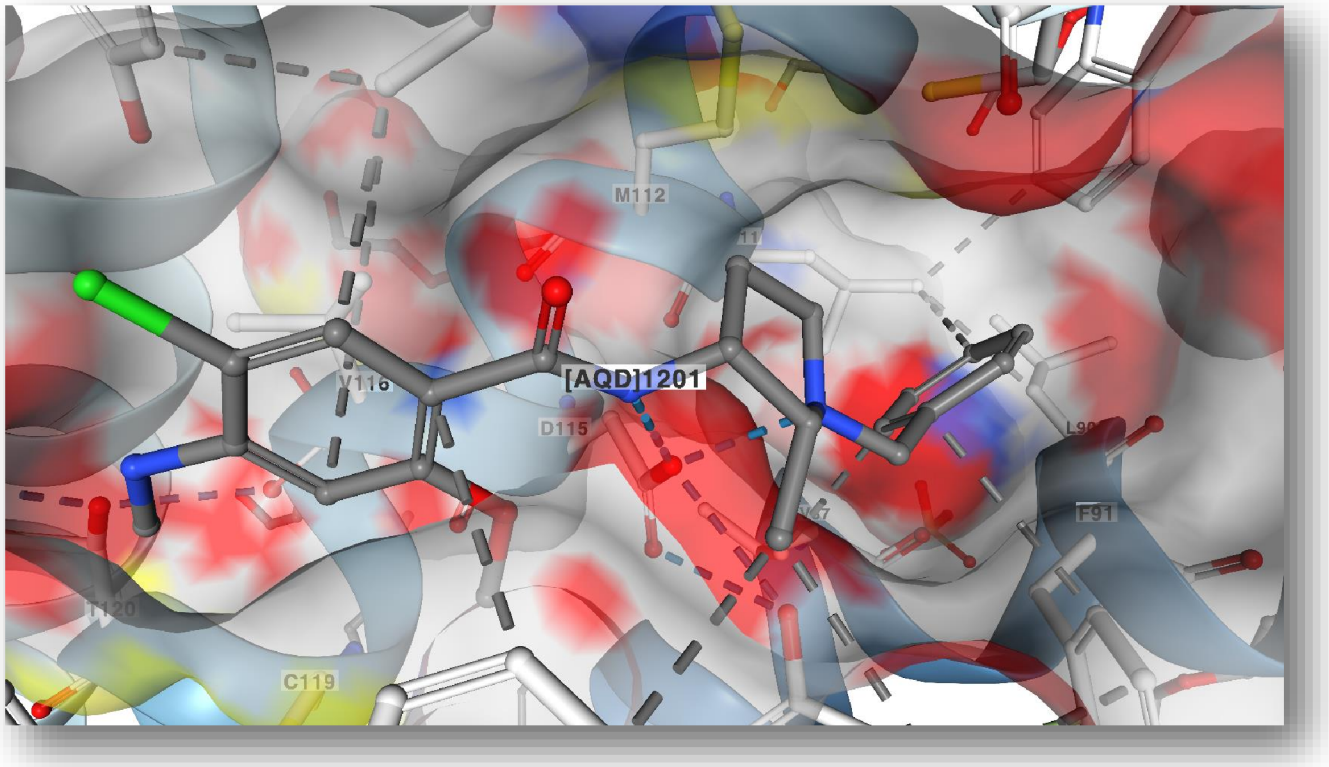
5 min 80 °C



[^{18}F]**5**

IN SILICO INHIBITOR DESIGN

Rational Inhibitor Design Based on Protein Databank Structure 5WIV

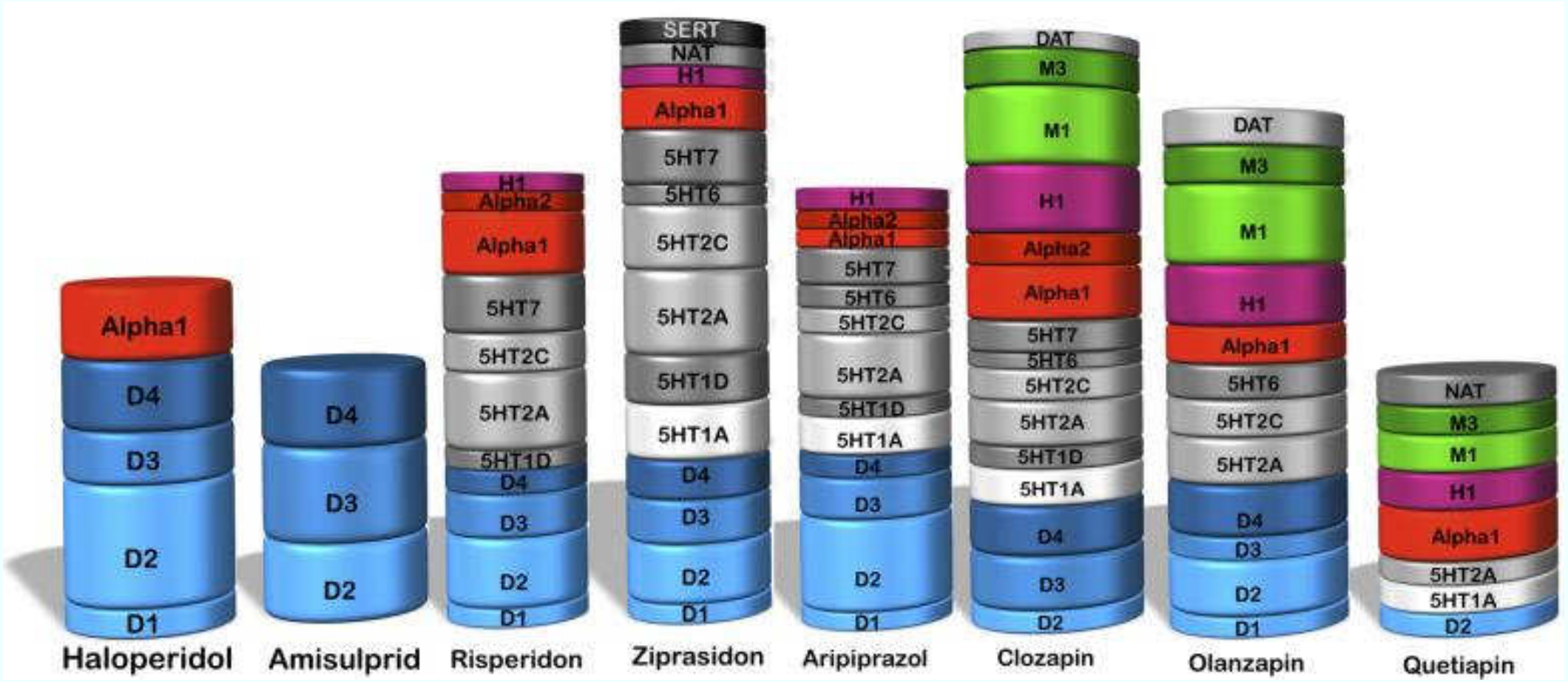


Structure of the sodium-bound human D4 Dopamine receptor in complex with Nemonapride (AQD). PDB Code: 5WIV

Structural information on available co-crystalized inhibitors can be leveraged

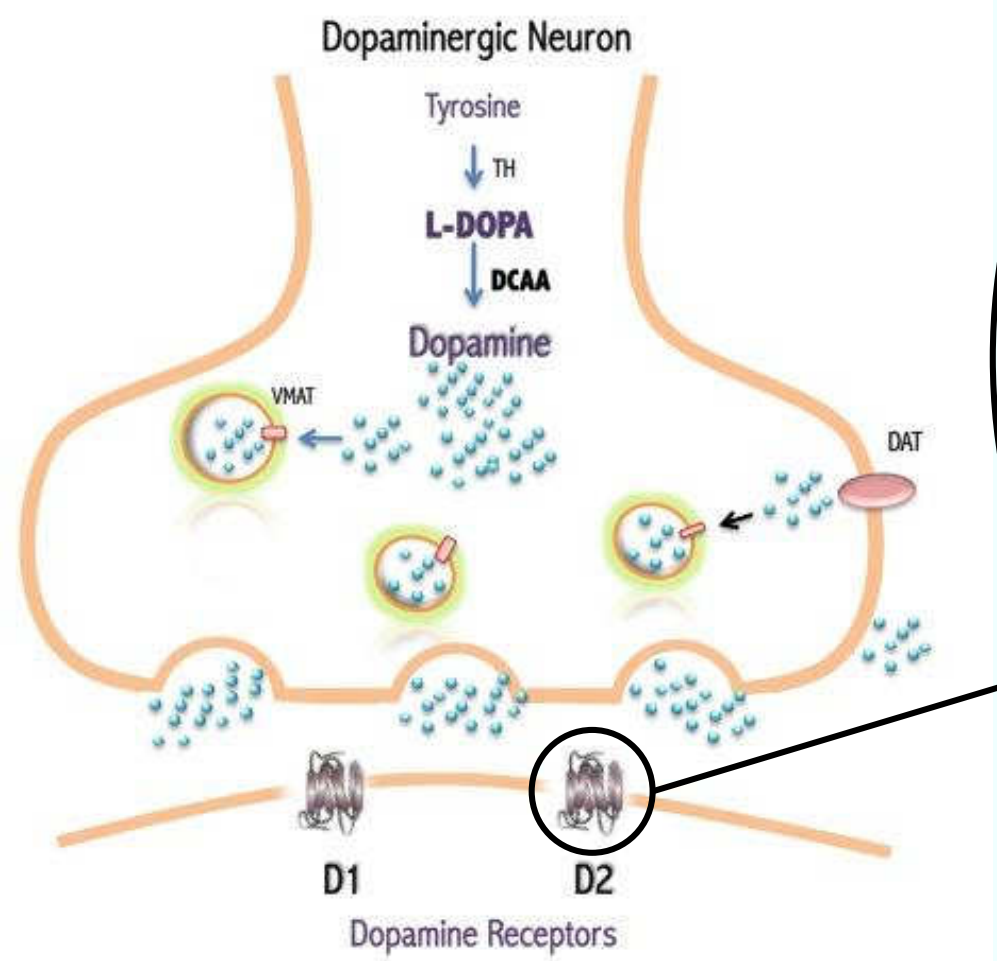
RECEPTORS ADRESSED BY NEUROLEPTICS

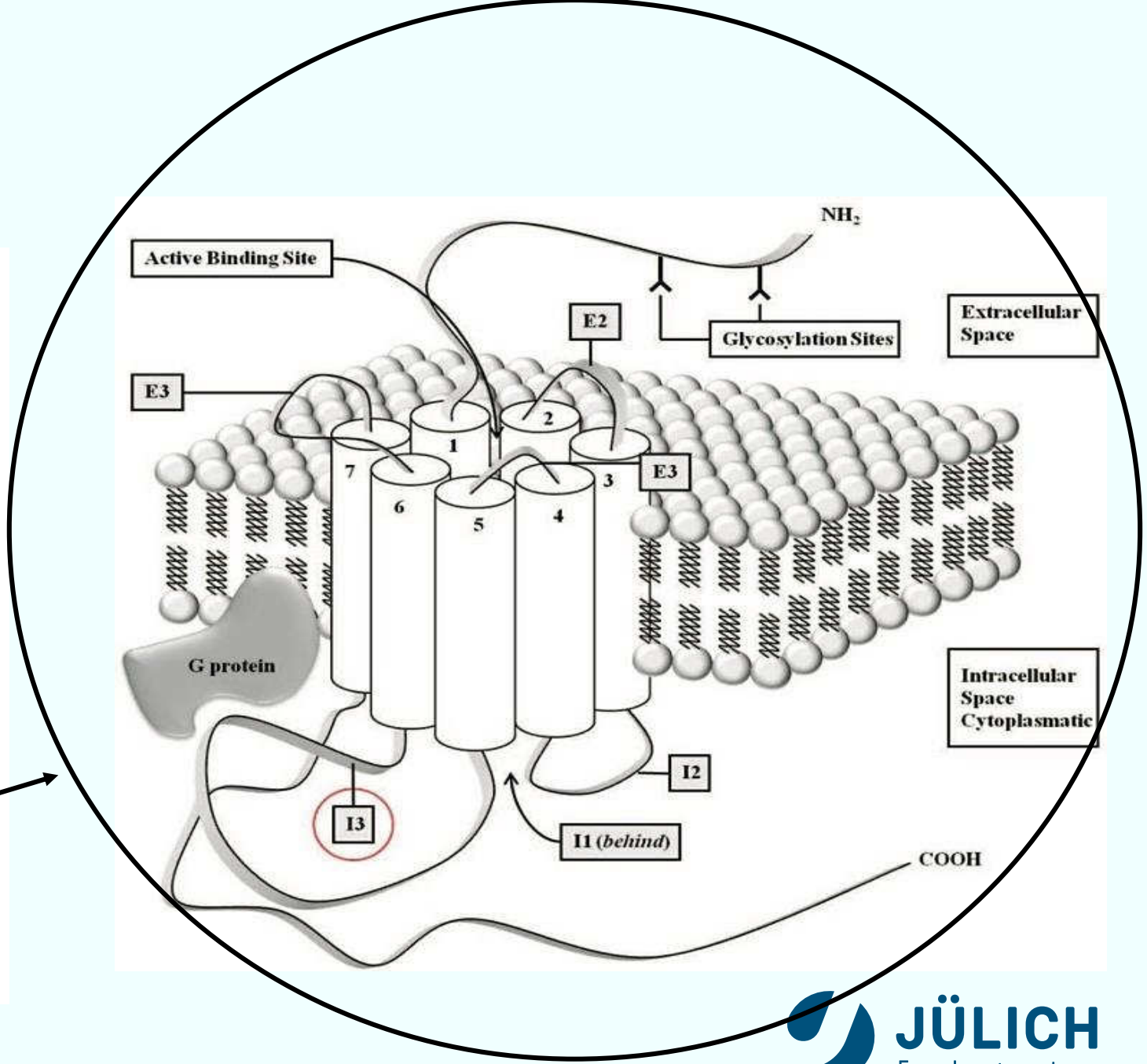
„Rich in Rharmacology“



DOPAMINERGIC SYSTEM

Dopamine

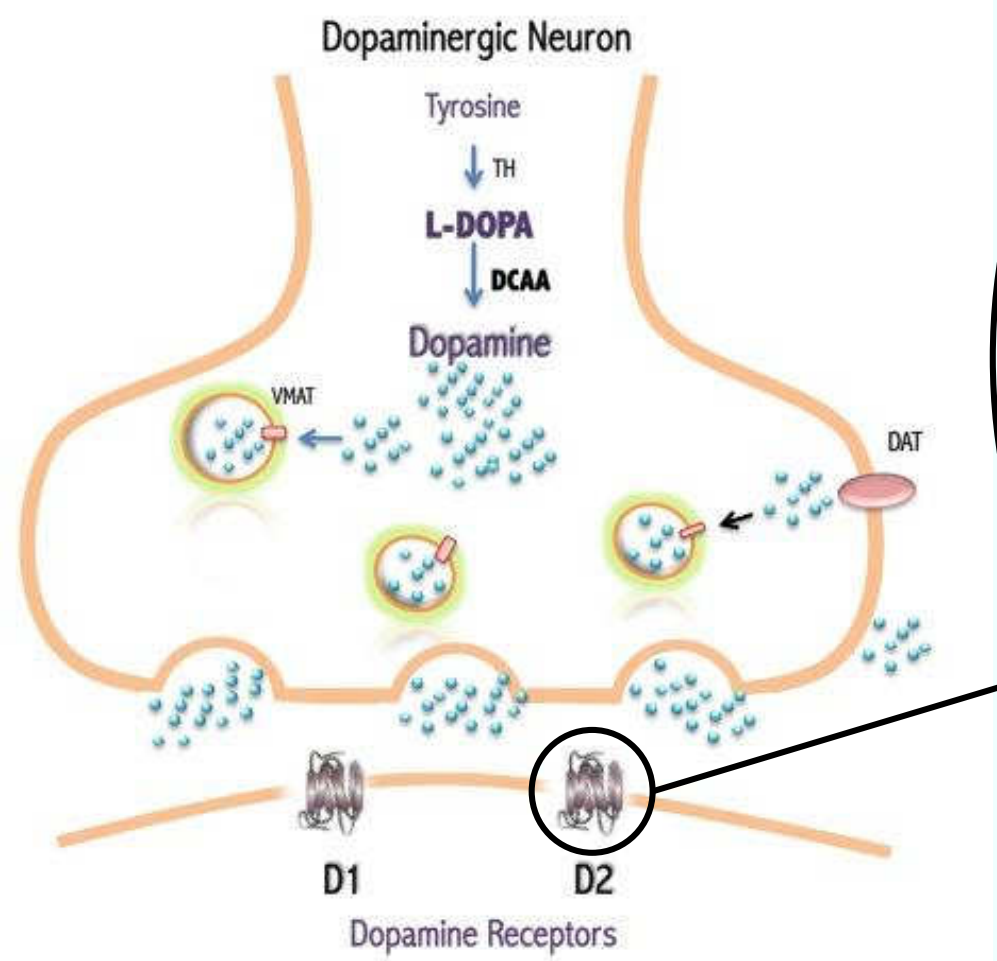


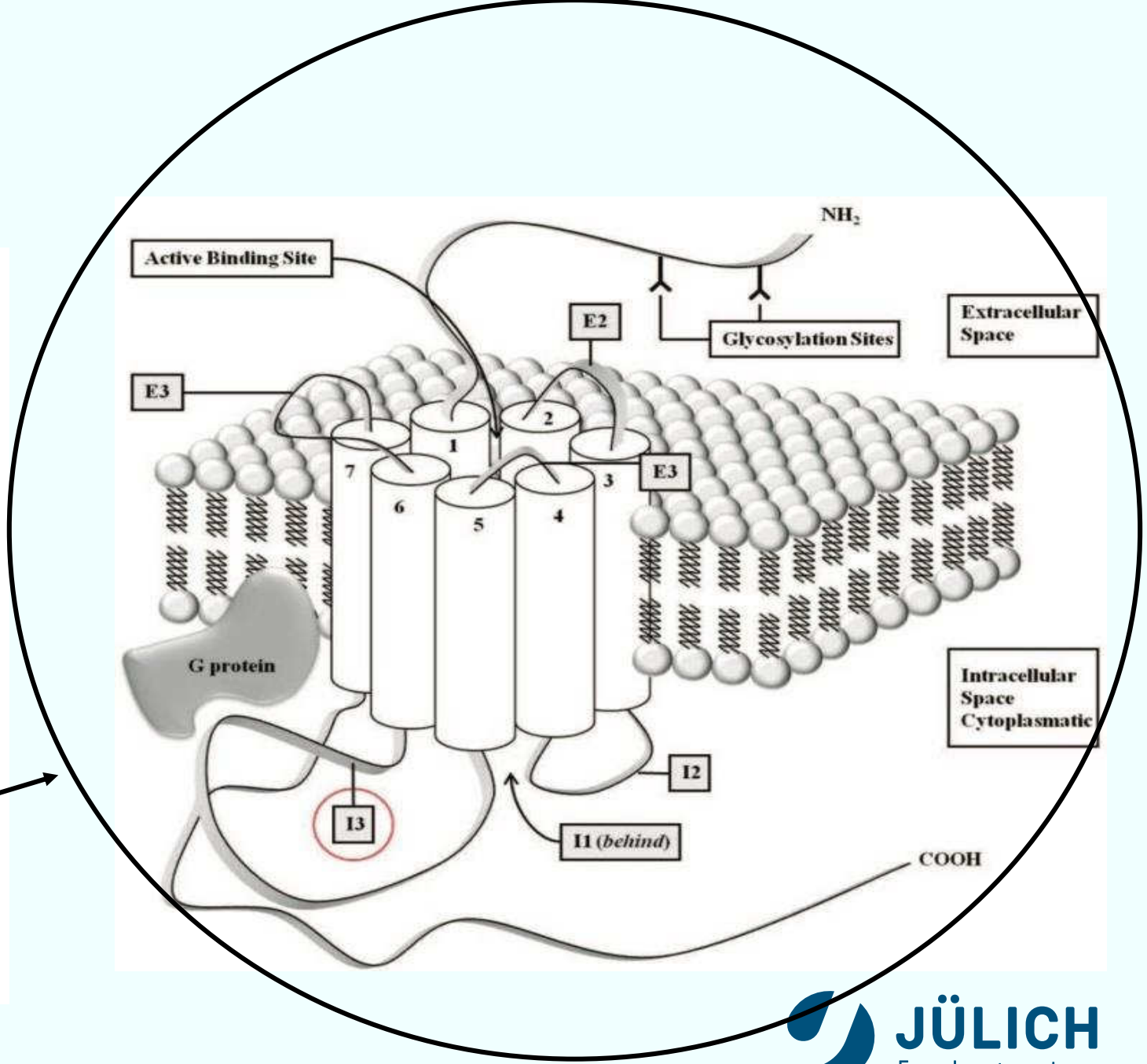


Primus, R. J., et al. (1997). Journal of Pharmacology and Experimental Therapeutics **282**(2): 1020-1027.
Santanavanich, C., et al. (2003). Journal of Pineal Research **35**(3): 169-176.

DOPAMINERGIC SYSTEM

Dopamine





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