UiT

THE ARCTIC
UNIVERSITY
OF NORWAY

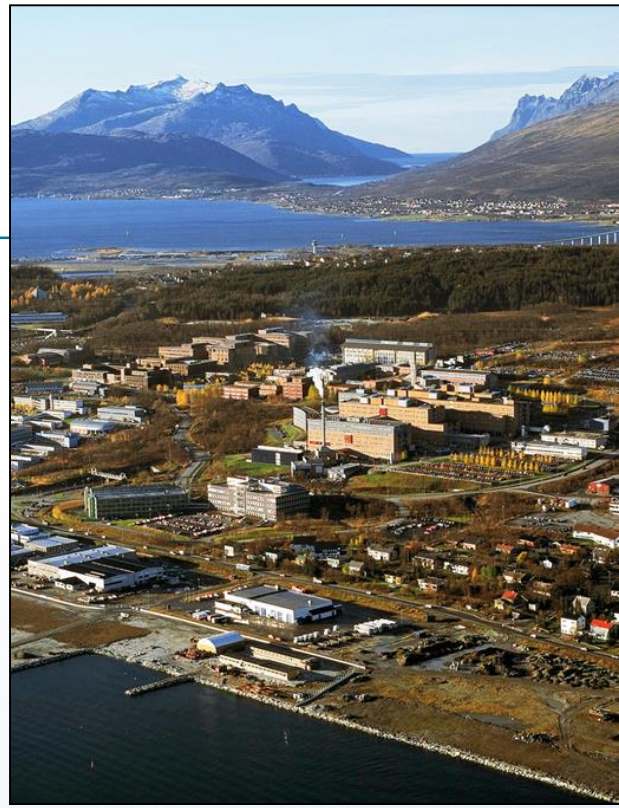
SCREENING FOR NEW SEROTONERGIC COMPOUNDS

Ingebrigt Sylte,
Department of Medical Biology,
Faculty of Health Sciences
UiT – The Arctic University of Norway

ICM User Group Meeting 2016, March 17-18 2016

UiT - The Arctic University of Norway

- UiT The Arctic University of Norway (former University of Tromsø)
- Students: 15 500
- Staff 3000
- Classic multi-disciplinary
- 69°40′N, 18°56′E
- Located at the same latitude as Siberia and Alaska

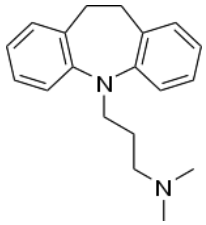


Outline

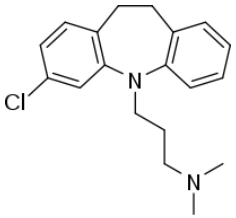
- Drug therapy of major depressive disorder
- The serotonin transporter (SERT)
- 4D docking to SERT homology models
- Virtual screening for new SERT/5-HT receptor compounds

Pharmacotherapy of major depressive disorder (MDD)

Tricyclic antidepressants (TCAs)

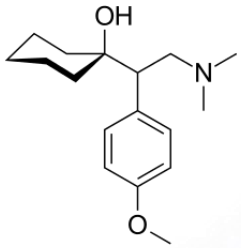


Imipramine



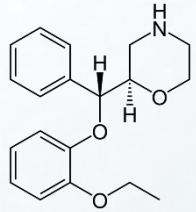
Clomipramine

Serotonin - noradrenaline reuptake inhibitors (SNRIs)



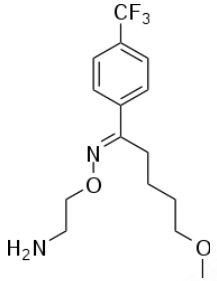
Venlafaxine

Noradrenaline reuptake inhibitors (NRIs)

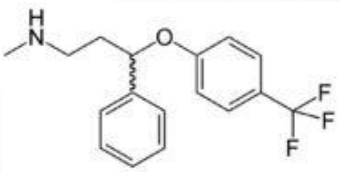


Reboxetine

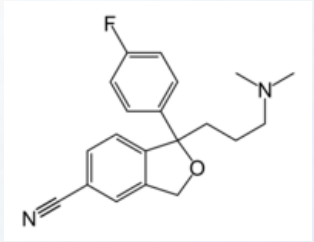
Selective serotonin reuptake inhibitors (SSRIs)



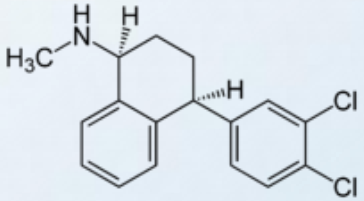
Fluvoxamine



Fluoxetine



S-citalopram

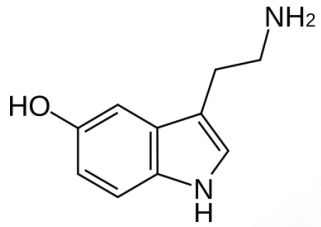


Setraline

Problems with SSRIs

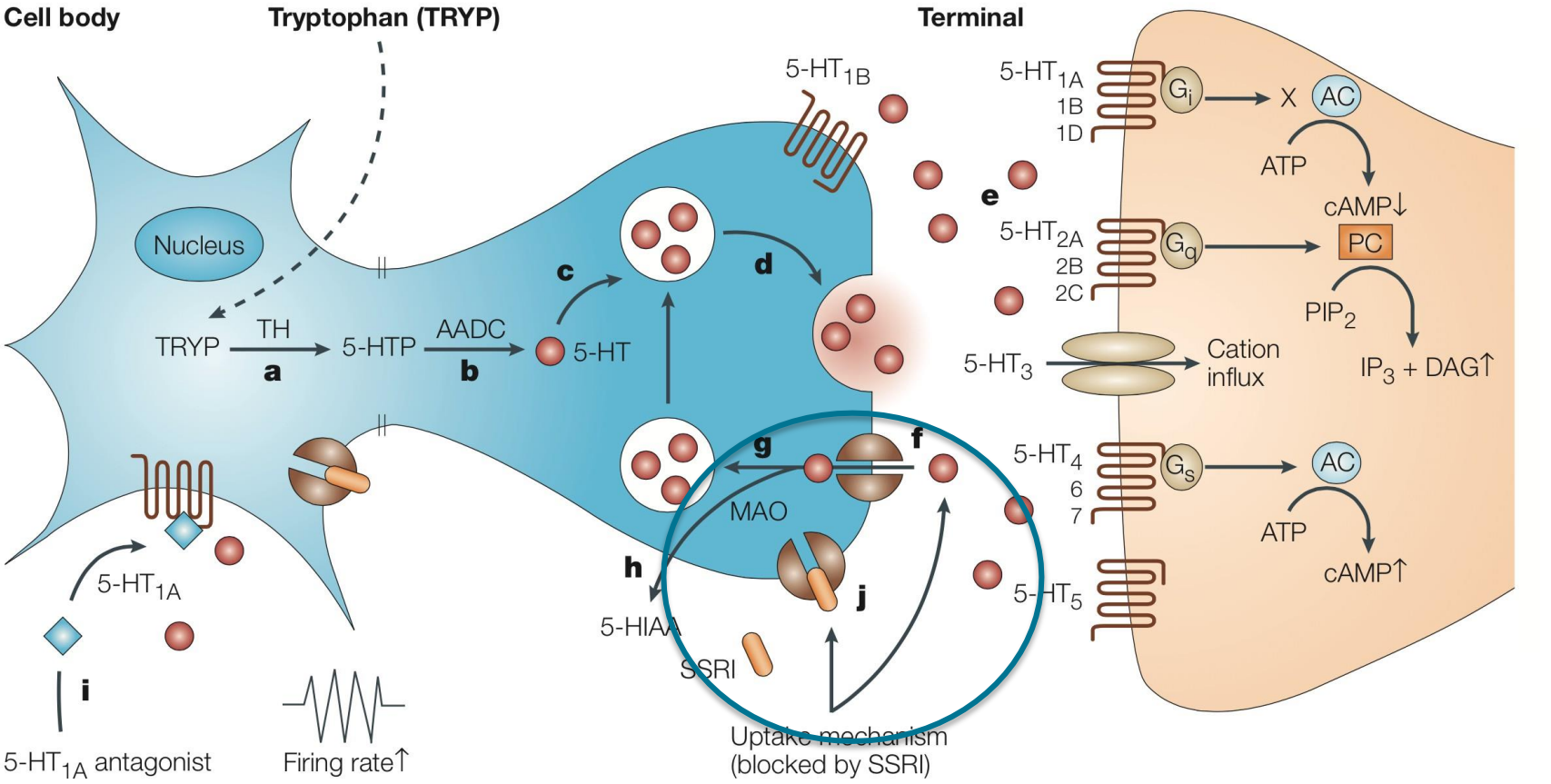
- Slow onset of action (3 to 6 weeks)
  - Ineffective for patients that need immediate relief
- Limited efficacy
  - Several patients do not respond to the treatment at all
  - Clinical trials with selected patient populations indicated response and remission rates between 40% to 60%
- Drug related adverse effects

- Consider new drug targets
- Multi-target or multi-modal compounds



Serotonergic neurotransmission

Serotonin = 5-hydroxytryptamine (5-HT)



Wong et al., Nature Reviews Drug Discovery 4, 764-774 (2005)

SERT – the serotonin (5-HT) transporter

Belongs to the NSS family of transporters

NSS: Neurotransmitter/sodium symporter family

Also known as the solute carrier family 6 (SLC6)

Located in the limbic areas of the brain

- Areas involved in processes such as mood, emotion and reward
- Closely related to the dopamine and noradrenalin transporters (DAT and NET)

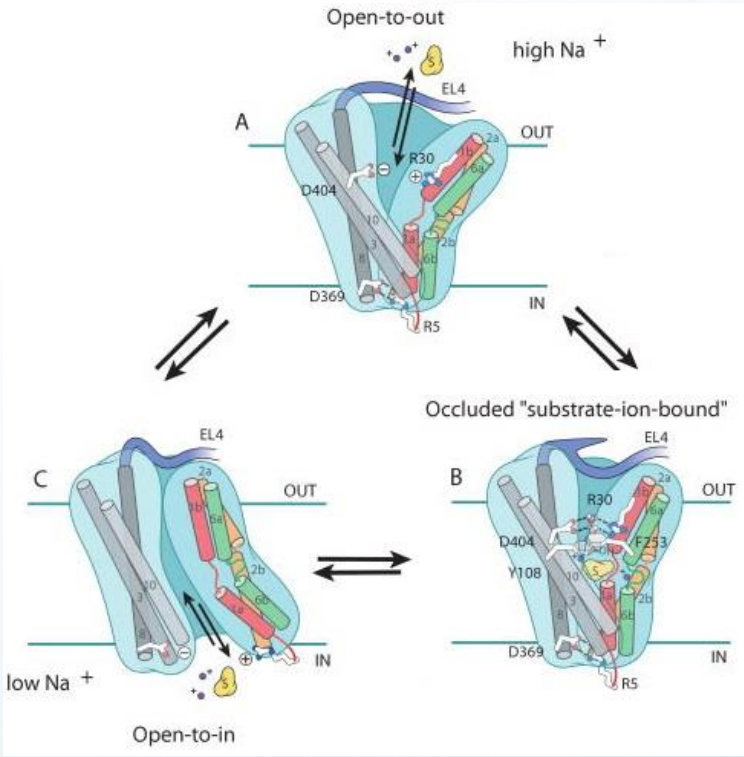
The 3D structure is not know.

LeuT structures as templates for SERT

The procaryotic *Aquifex aeolicus* leucine transporter (LeuT)

- Approximately 20 % sequence identity to eukaryotic NSSs.
- Outward open conformation
  - S1site accessible from extracellular region.
  - Competitive inhibitor Trp[1].
- Outward-occluded
  - Substrate[2] in S1, SSRIs[3] (non-competitive inhibitors) in S2 site
- Inward open
  - S1 site accessible from cytoplasma
  - Apo inward-open[4]

Alternate-access transport mechanism[1]



1. Singh et al. 2008, Science, 322, 1655-1661. 2. Piscitelli et al. 2010, Nature, 468, 1129-1132. 3 Zhou et al. 2007, Science, 317, 1390-1393.
4. Krishnamurthy and Gouaux, Nature, 2012, 481, 469-474

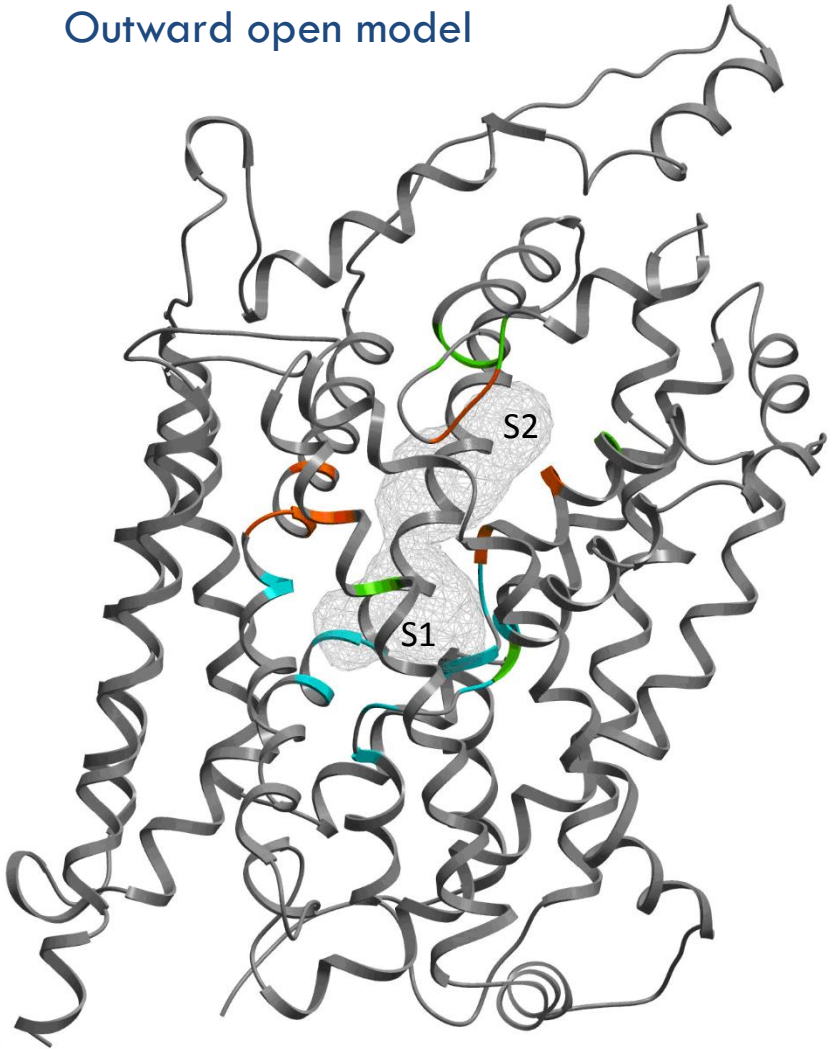
Is it possible to use SERT homology models based on LeuT templates for studying SERT – inhibitor interactions

- Most SSRIs and TCAs are competitive inhibitors of SERT
- Site directed mutagenesis data indicate that inhibitors interact with SERT via amino acids in S1
- D98 (S1-site) of SERT is a key residue attracting positively charged ligands
- SSRIs and TCAs interact with D401 in S2 of LeuT which correspond to K490 in SERT.
- A negatively charged amino acid is lacking in the S1 of LeuT.

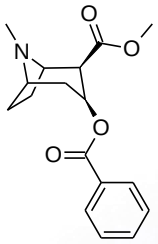
- Can we use LeuT as a SERT template for studying the interactions with antidepressants?

Docking into SERT models

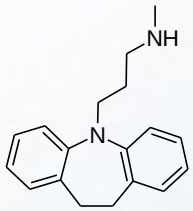
Outward open model



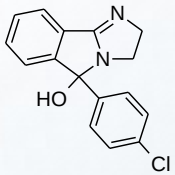
58 compounds from 5 classes



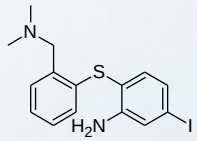
Cocaine (3-phenyltropanes)



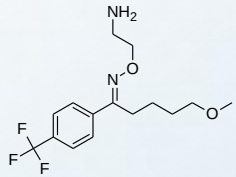
Desimipramine (TCAs)



Mazindole (Mazindoles)

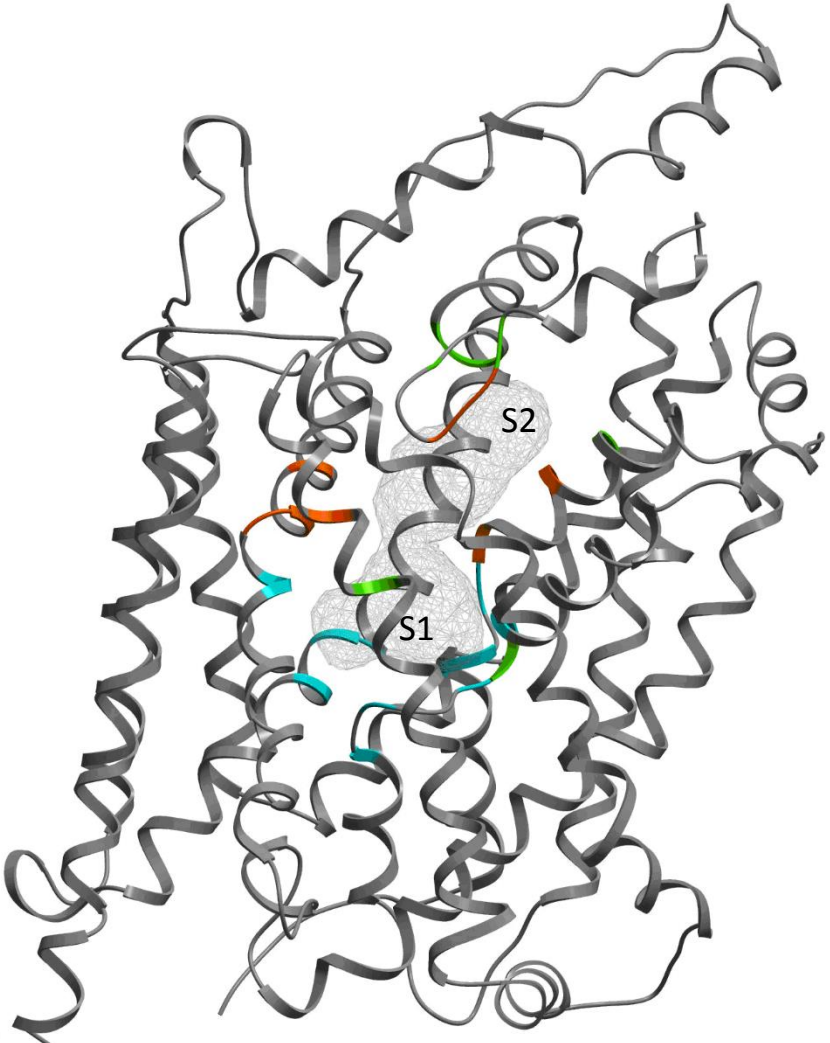


ADAM (SERT radioligands)



Fluvoxamine (SSRIs)

Outward open model – 4D docking



Flexible docking protocol

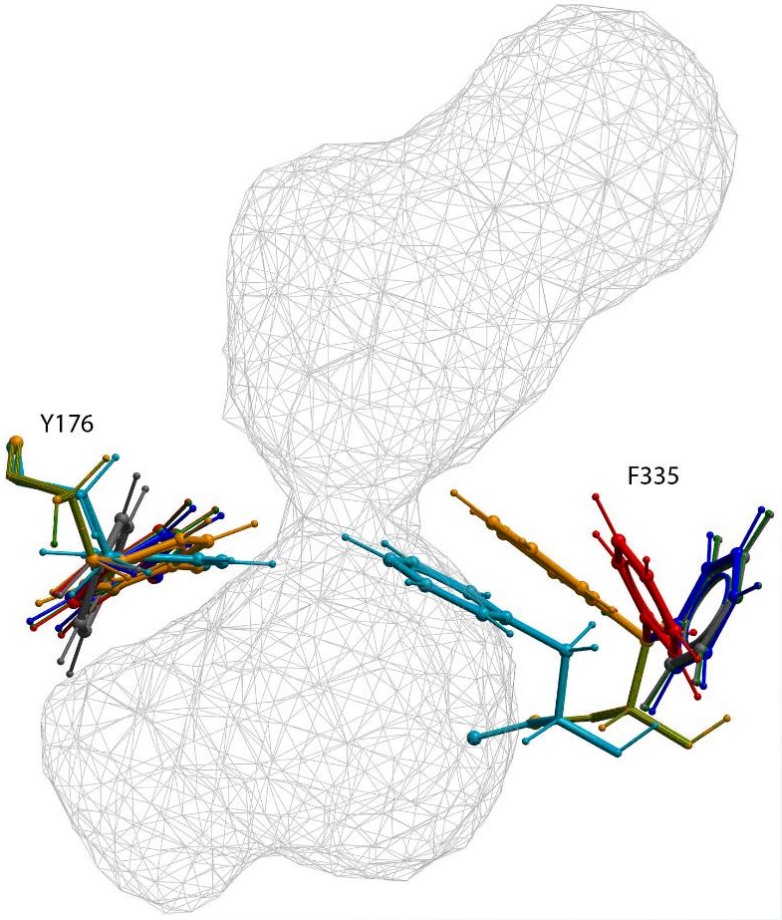
1. Selecting binding pocket.

2. Biased-probability Monte Carlo (BPMC) sampling and minimization of the pocket side chains in the presence of a repulsive density representing a generic ligand to prevent collapse of the binding pocket.

3. 4D docking, docking of flexible ligands into the ensemble of binding site conformations.

Fumigation

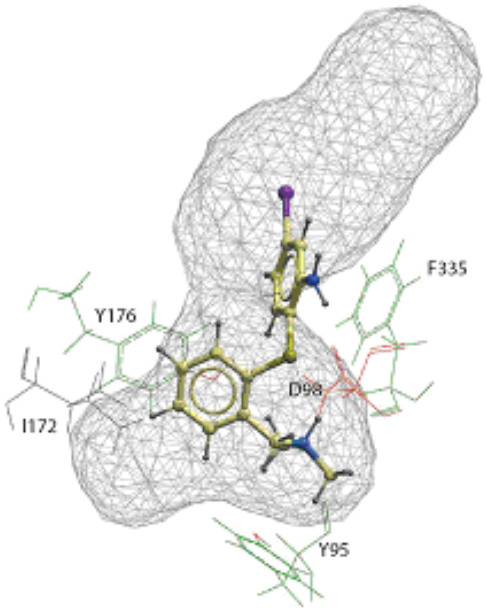
- opening of the extracellular gate



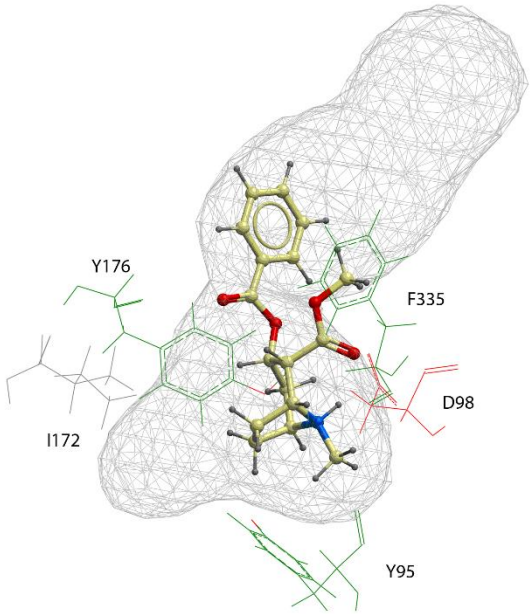
Shortest distance (Å) Y176-F335	Conformation
4.4	Initial
12.6	18
11.9	23
8.7	24
12.1	32

Gabrielsen et al. 2012, Eur. J. Med.Chem. 47(1), 24-37.

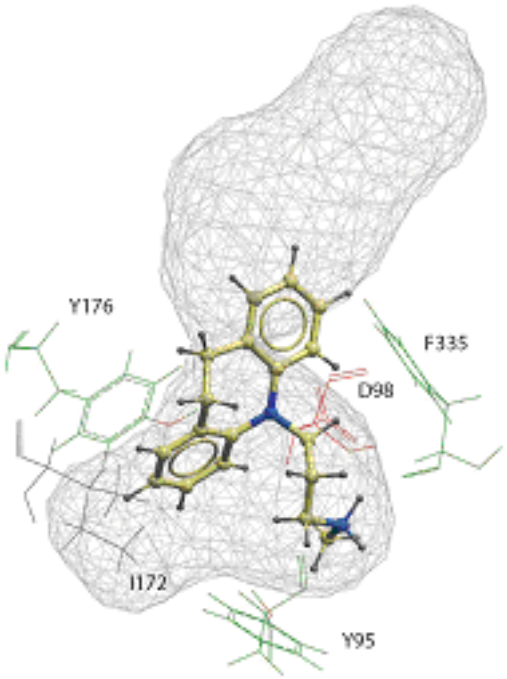
ADAM, conf. 18



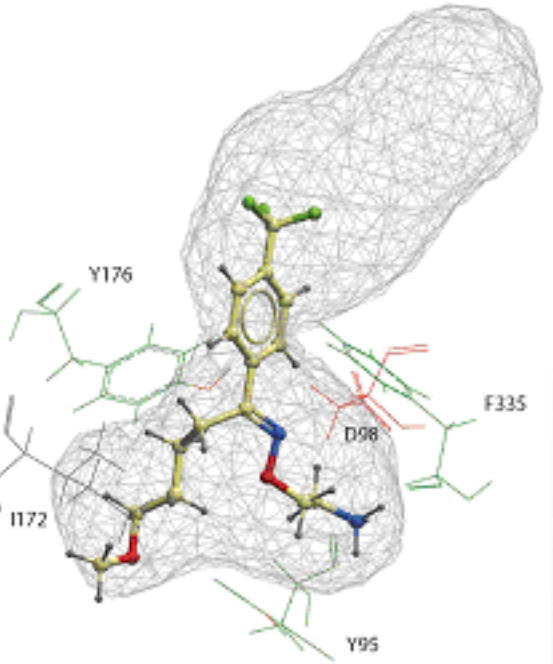
Cocaine, conf.2



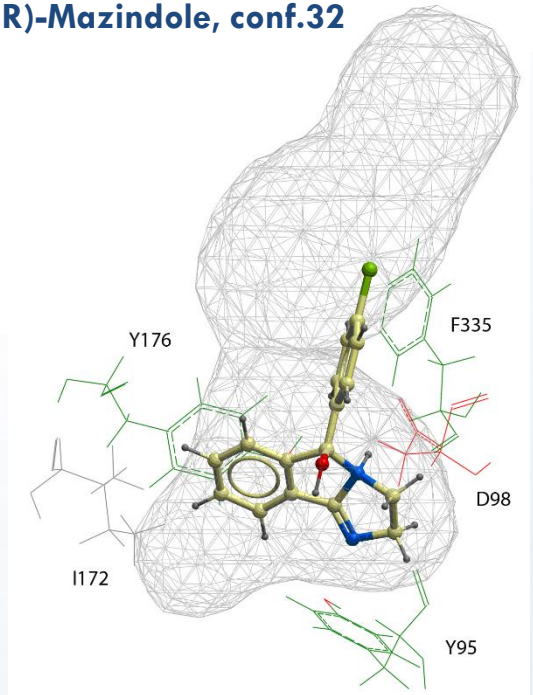
Desimipramine, conf. 24



Fluvoxamine, conf.5



(R)-Mazindole, conf.32



- TCA and SSRIs bind in S1

Gabrielsen et al. 2012, Eur. J. Med.Chem. 47(1), 24-37.

Recent X-ray structures - SERT templates

LeuTBAT

- LeuT where key binding site amino acids within 12 Å of the Trp were mutated to hSERT[1].
- In complexed with *4* different structural classes of SSRIs
- Bind in the S1-site

Drosophilia DAT

- In complex with nortriptyline[2].
- In complex with dopamine, substrate analogues and stimulants[3].
- In complex with different SSRIs and NRIs[4]
- Bind in the S1-site

1. Wang et al. 2013, Nature, 503, 141-146. 2. Penmatsa et a. 2013, Nature, 503, 85-91.
3. Wang et al. 2015, Nature, 521, 322-328. 4. Penmatsa et al. 2015, Nature Struct Mol Biol, 22(6), 506-508.

Multistep combined virtual screening (VS) protocol

3.24 million drug like compounds

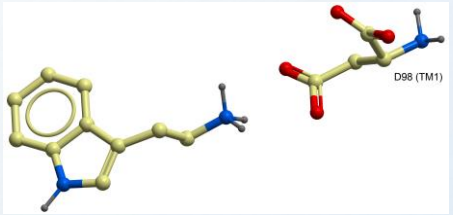
1. Ligand based approach
- 2D fingerprints based on the reference compounds
- Basic property filter
- ADMET filtering
- 3D Pharmacophore fitting

2. 4D docking into the 47 active site conformations from Fumigation

3. *In Vitro* evaluation of hits
- [^{3}H]-citalopram/rat neocortical tissue

4. Substructure search using the core structures of 13 chemotypes with ki > 1000 nM.

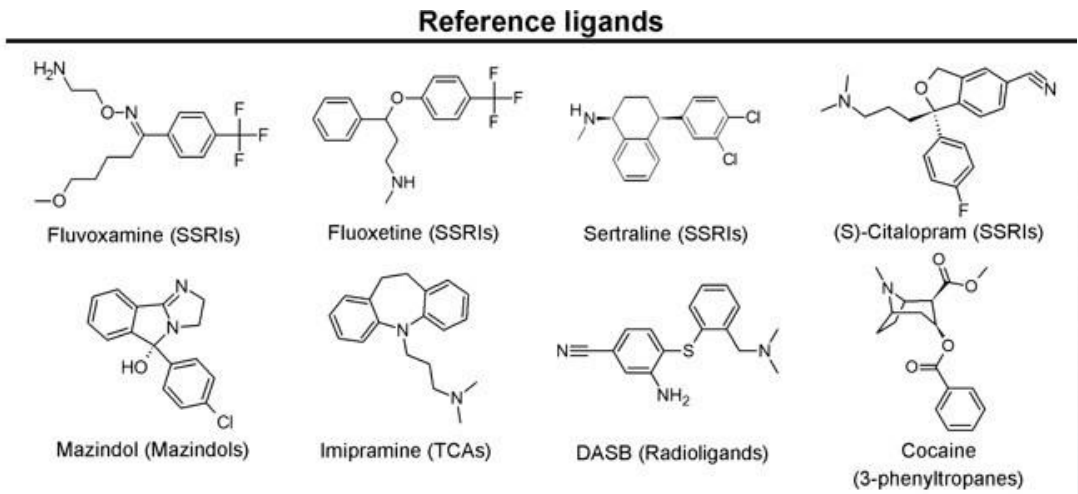
5. Repeating steps 1 - 3

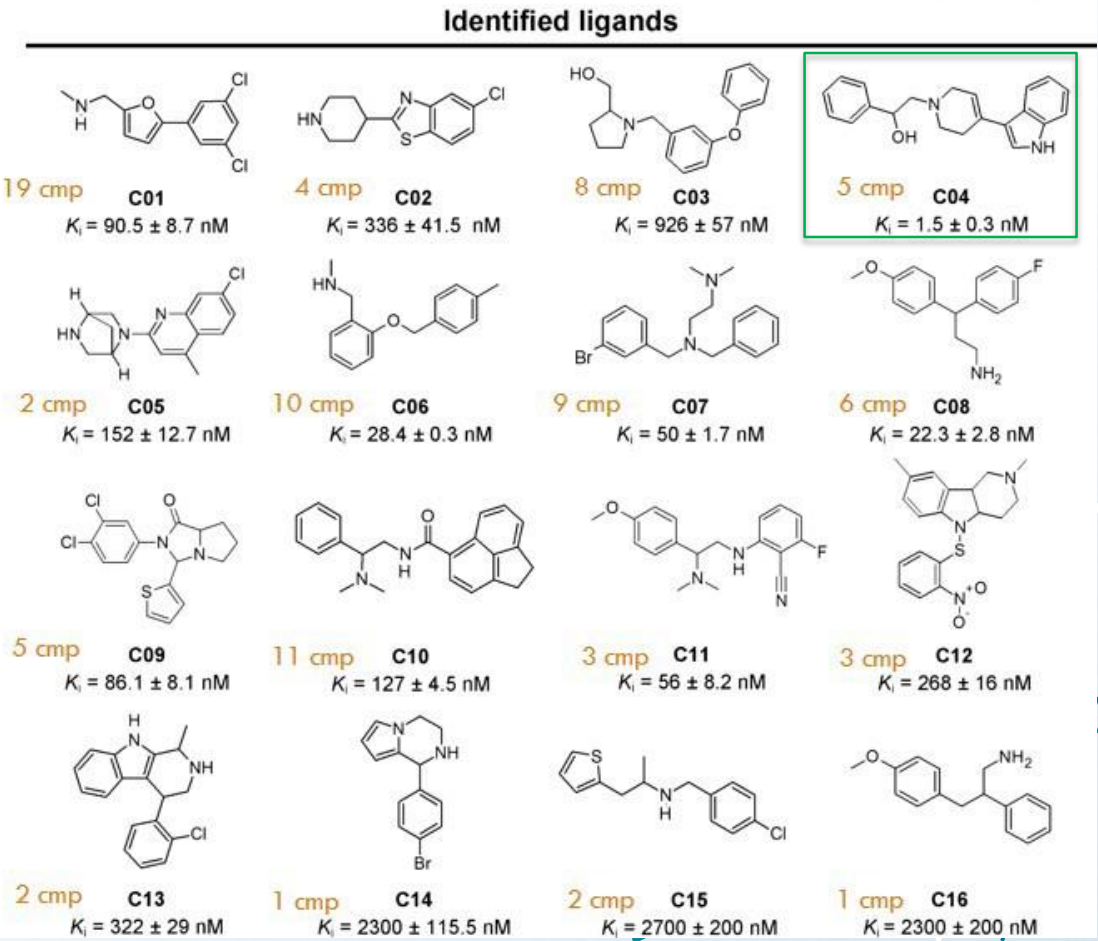


Gabrielsen et al., J Chem Inf Model (2014),54(3):933-43

In vitro evaluation

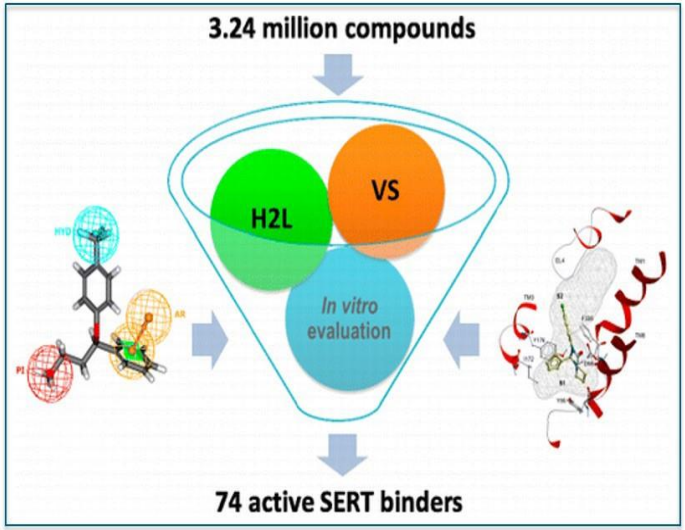
- In vitro evaluation
  - 400 compounds: screening
  - 97 compounds: full binding studies
- Identified compounds
  - 74 compounds: $K_i \leq 5000$ nM
  - 46 compounds: $K_i \leq 1000$ nM
  - Substructure search resulted in ligands with higher affinity than their 'parent' compounds in 5 chemotypes





Gabrielsen et al., J Chem Inf Model (2014),54(3):933-43

Affinity for 5-HT receptors



46 compounds - affinity ≤ 1000 nM

Ki values (nM)

Compound	SERT	5-HT$_{1A}$	5-HT$_{2A}$	5-HT$_{6}$	5-HT$_{7}$
7	91			292	
11	2	56	217	569	314
14	107		508		
18	22				
25	164		839	117	730
28	288	8		101	126
30	43				
31	28			742	819
35	50			596	
36	84		265	856	830
45	268			69	
46	790		315	46	92

5-HT$_{1A}$: 4 ≤ 1000 nM, 5-HT$_{2A}$: 11 ≤ 1000 nM, 5-HT$_{6}$: 12 ≤ 1000 nM, 5-HT$_{7}$: 8 ≤ 1000 nM

Conclusions

- Found new SERT compounds
  46 with Ki values ≤ 1000 nM.
- Some of the compounds with relatively high affinity for 5-HT receptors.

- Build a multistep combined VS protocol that function for SERT.
- Homology based models (approx. 20 % sequence identity) were useful for the structure based step.
- **Fumigation and 4D docking were necessary for a successful result**.
- Predicted binding of TCA and SSRIs into S1 site, later supported by experimental structures.

Acknowledgements

- **National Medicine Institute, Warsaw**
  Malgorzata Jaronczyk
  Zdzislaw Chilmonzcyk
- **Polish Academy of Sciences, Krakow**
  Rafal Kurczab
  Gabriel Nowak
  Andrzej Bojarski,
  Andrzej Pilc
- **Skaggs School of Pharmacy and Pharmaceutical Sciences, San Diego**
  Irina Kufareva
  Ruben Abagyan
- **UiT The Arctic University of Norway**
  Mari Gabrielsen,
  Kurt Kristiansen,
  Aina W. Ravna
  Isak A. Bøgwald

POLISH-NORWEGIAN RESEARCH PROGRAMME

The Research Council of Norway