Molecular Modeling of Serotonin and Its Transporter

Jaturong PRATUANGDEJKUL

OUTLINE

I. Serotonin (5-HT)

II. Serotonin Transporter (SERT)

III. 3D-QSAR Analysis and Pharmacophore Definition of SERT-uptake

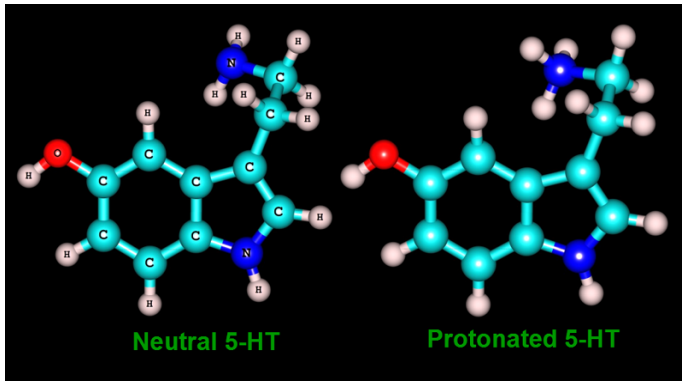
IV. Serotonin Intramolecular Cation-π Interactions

V. Conformational Dependence of 5-HT Theoretical pK_a Calculation

VI. Future Perspectives

I. SEROTONIN (5-hydroxytryptamine, 5-HT)

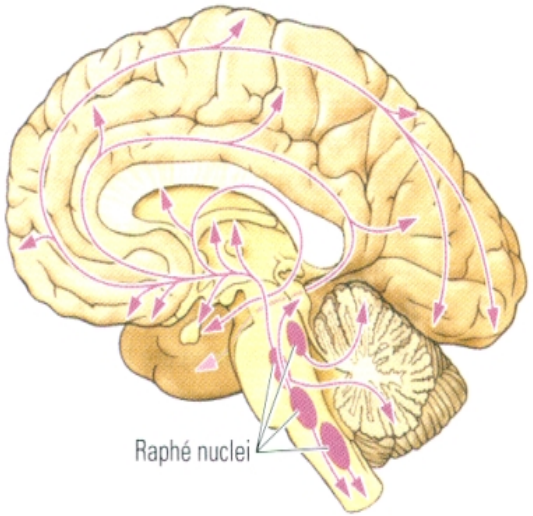
Structure



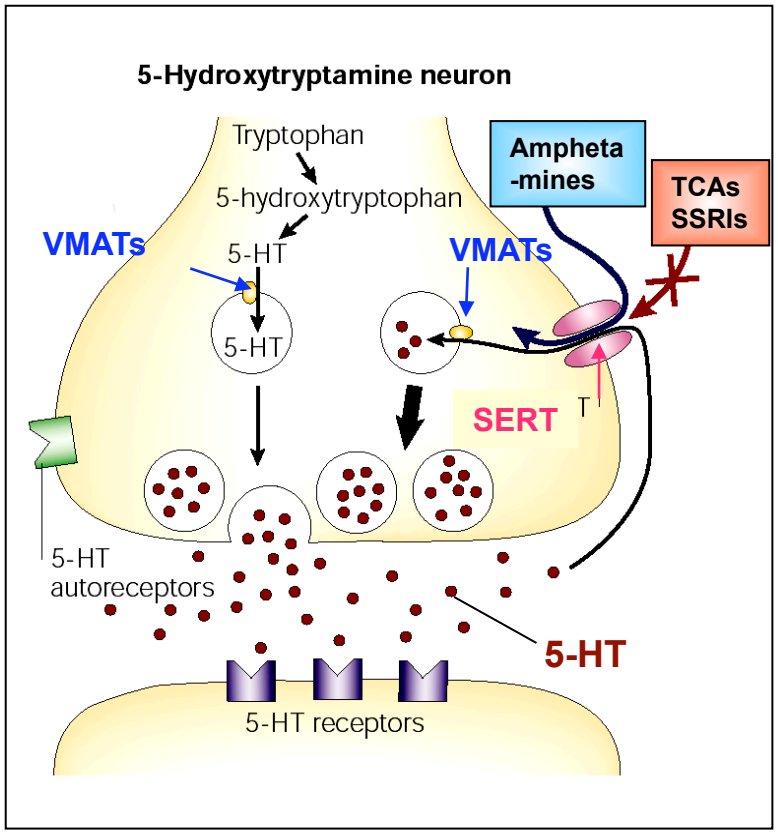
- $C_{10}H_{12}N_2O$
- IUPAC name: 3-(2-aminoethyl)-1*H*-indol-5-ol
- MW 176.22 g/mol
- Complex with creatinine sulfate monohydrate, and other salts e.g.hydrogen oxalate, picrate monohydrate, hydrochloride
- pK_{a1} = 9.97, pK_{a2} = 10.73
- Symmetric or achiral molecule, no optical isomers

Distribution

- 5-HT cells → 90% in enterochromaffin cells of gut,
- 5-HT cell bodies → *raphe nuclei* of brainstem, project to cortex as well as to cerebellum and spinal cord
- 5-HT → high concentration stored in dense granules of ***platelets***

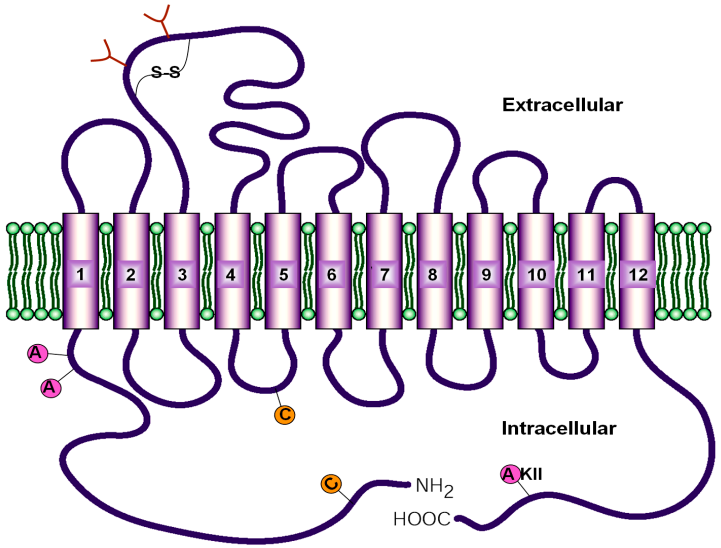


Storage, Release & Re-uptake



II. 5-HT TRANSPORTER (SERT)

- Na^+/Cl^--dependent transporter family
- Co-transport of external Na^+ and Cl^- and counter-transport of K^+ (electroneutral)
- 12 TMDs, large EL between TMD3 & TMD4 with N-linked glycosylation sites
- N- and C-termini in cytoplasm with phosphorylation sites
- hSERT spans 37.8 kb on chromosome 17q11.2, encodes 630 amino acids.

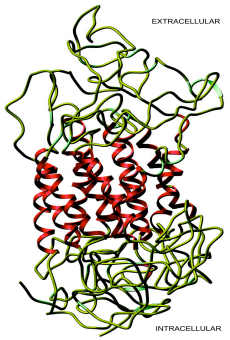


Computational Approaches for Molecular Modeling of SERT

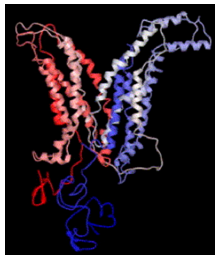
Homology Modeling Approach

3D-SERT model

NhaA as template[1]



LacY as template[2]



Need to evaluate the reliability of those SERT-models by experiment
(*e.g. site-directed mutagenesis & ligand-binding assays*)

Ligand-based Approach

3D-QSAR model & definition of pharmacophore for compound-interacting SERT
(*when bioassays data of different substrates or inhibitors are available*)

Database searching & prediction of activity

Evaluate the reliability of 3D-QSAR model & pharmacophore by bioassays

[1]Ravna *et al., J. Pharmacol. Exp. Ther.* (**2003**), 307, 34.
[2]Ravna *et al., Bioorg. Med. Chem. (**2005**) (published on line)*

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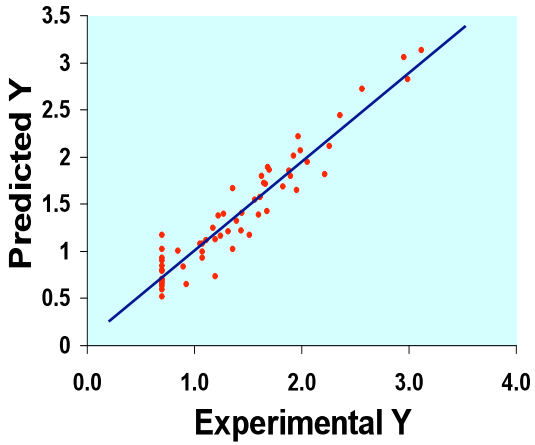
III. 3D-QSAR ANALYSIS & PHARMACOPHORE DEFINITION OF SERT-UPTAKE

QSAR : quantitative structure-activity relationship

$$Y = a_1X_1 + a_2X_2 + a_3X_3 + \ldots.a_nX_n + \text{constant}$$

where Y = dependent variable (biological activity)
X = independent variable (structural property → "descriptor")
a = coefficient



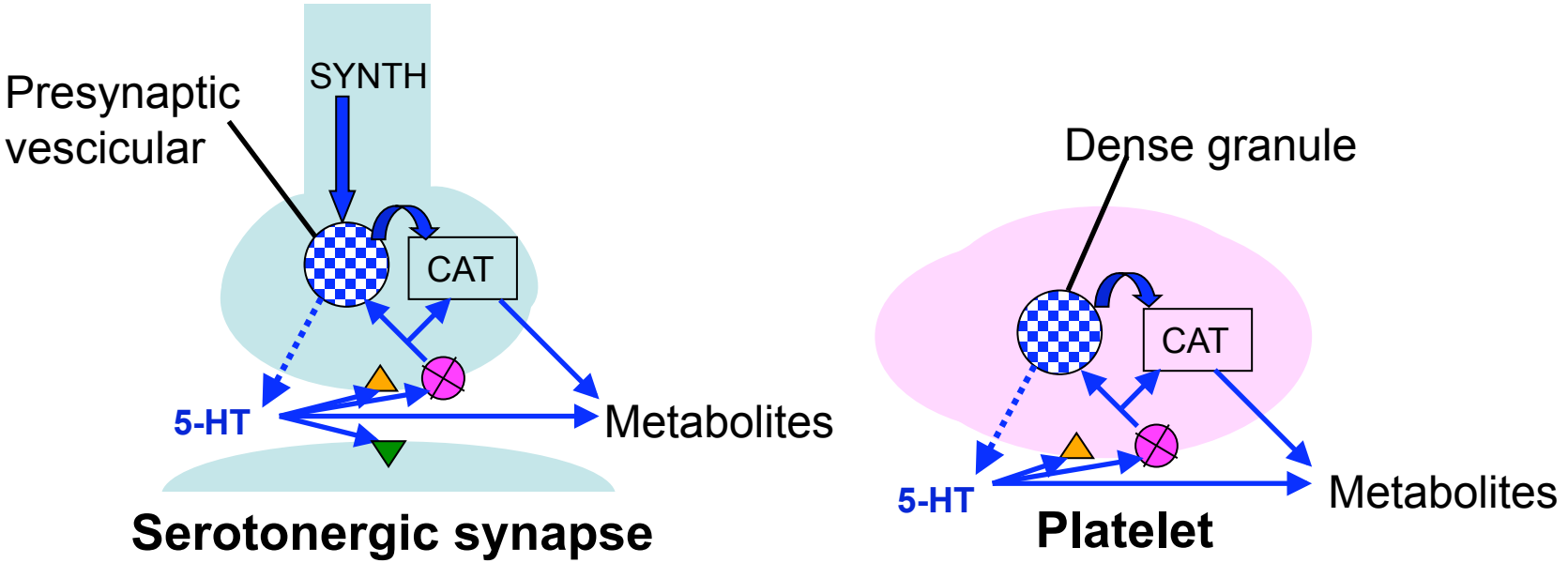
***In silico* 3D-QSAR**
of the initial compound library, in order to define the physicochemical properties of molecules transported through SERT.

The best 3D-QSAR model of SERT transport activity enabled us to define a reliable **pharmacophore of SERT-uptake**.

Preparation of Input Data

121 compounds library:
- 5-HT analogs
- Harmanes
- Benzothiazoles
- Indanones
- Amphetamine derivatives

Assay of SERT-uptake: through SERT in human blood platelets



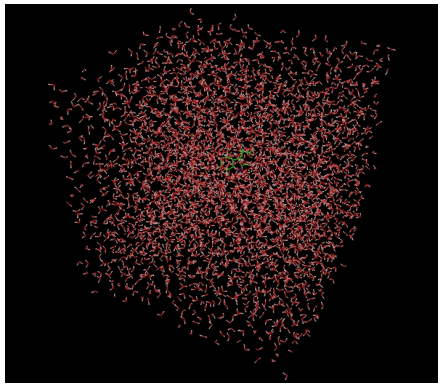
Storage Re-uptake Pre-synaptic receptor Post-synaptic receptor

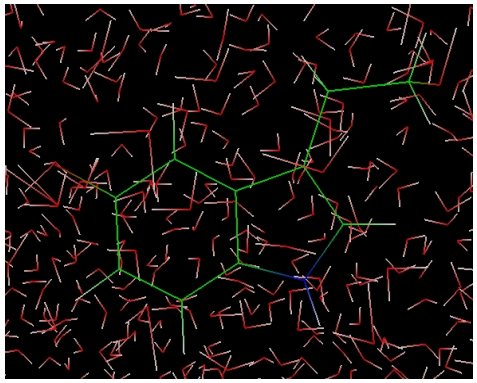
Release CAT Catabolism SYNTH Synthesis

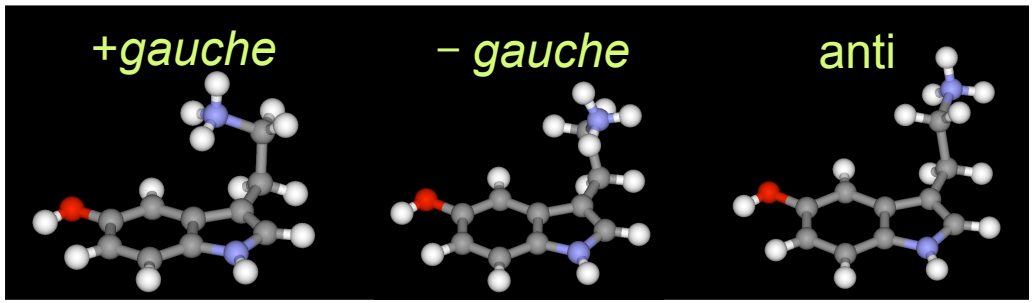
3D Structure Optimization

Conformational Analysis

Minimization &
Molecular Dynamics



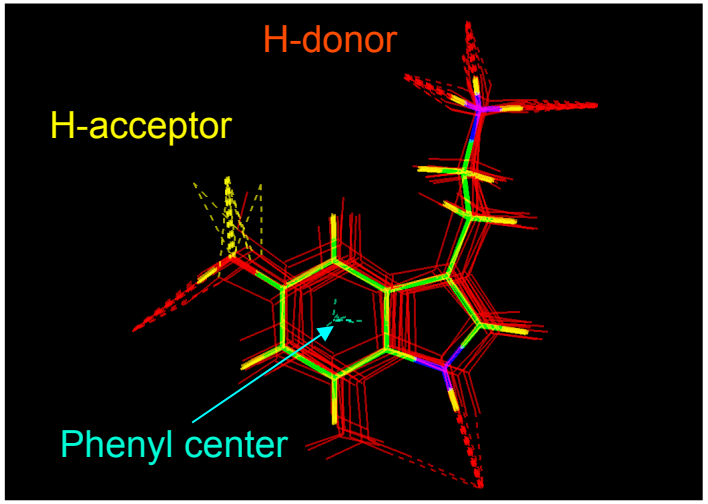


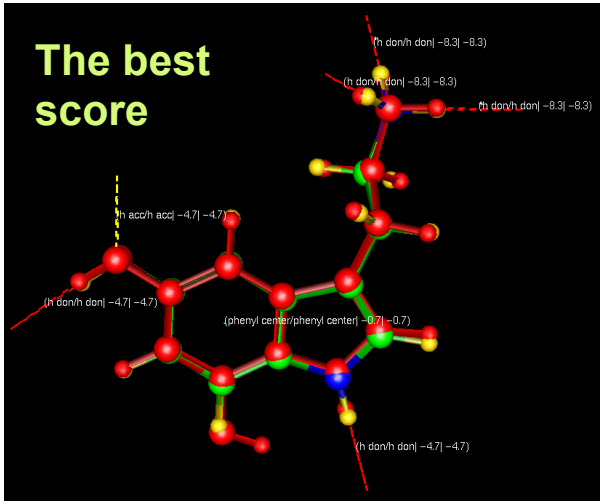


Establisment of Bioactive Conformation

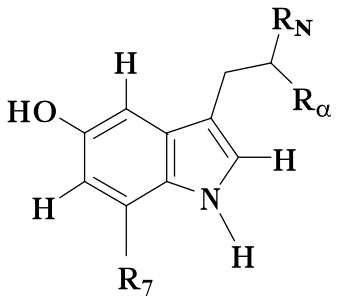
Ab initio conformational analysis of 5-HT at the B3LYP/6-31+G(d,p) level of theory

Alignment of Structures

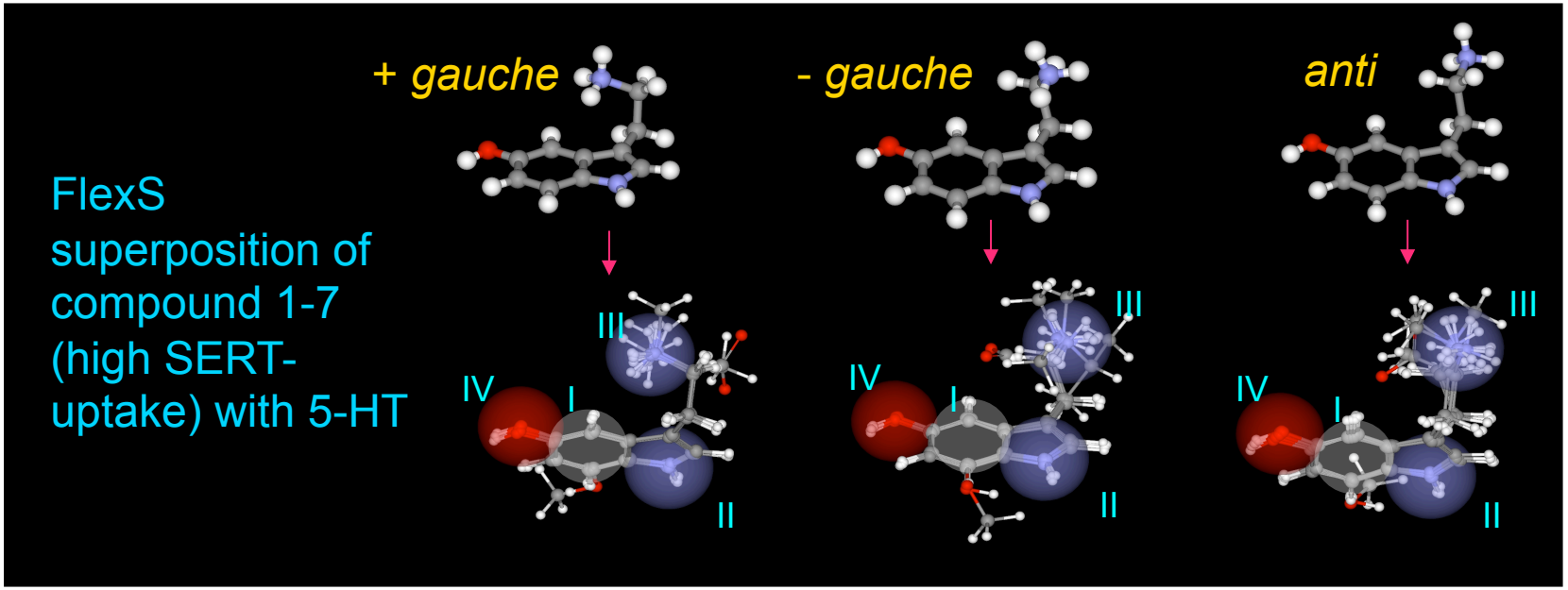




7 most active compounds



Compound	R_6	R_7	R_α	R_N	Uptake[(a)]
1	H	H	H	NH_3^+	13.31 ± 0.27
2	H	H	H	$NH_2^+(CH_3)$	9.87 ± 0.12
3	H	H	CH_3	NH_3^+	9.21 ± 0.05
4	H	H	H	$NH^+(CH_3)_2$	3.75 ± 0.64
5	H	OCH_3	H	NH_3^+	1.84 ± 0.09
6	H	OH	H	NH_3^+	1.66 ± 0.04
7	H	H	COO^-	NH_3^+	1.15 ± 0.08



4 important structural determinants

I = Phenyl ring center of the indole structure
II = Indole nitrogen atom
III = Ammonium group of the ethylamine side chain
IV = R_5 hydroxyl group

QSAR of SERT-uptake

$$Y = a_1X_1 + a_2X_2 + a_3X_3 + \ldots..a_nX_n + \text{constant}$$

where Y = SERT-uptake activity
X = descriptor
a = coefficient

Classical QSAR

46 analogs of 5-HT

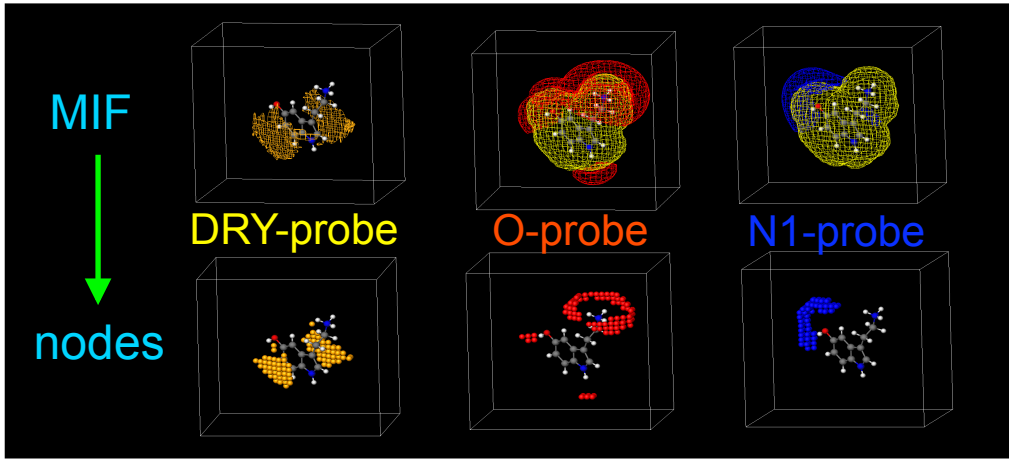
Statistical parameters	Mod I(*t*)	Mod II(-*g*)	Mod III(+*g*)
No. of descriptors	22	22	22
No. of LVs	2	2	2
LOO-cross-validation			
$q^2_{(LOO)}$	0.2434	0.2232	0.2266
$s_{(LOO)}$	0.8600	0.8726	0.8702
LFO-cross-validation			
$q^2_{(LFO)}$	0.3011	0.2446	0.27297
$s_{(LFO)}$	0.8272	0.8601	0.8437

Included descriptors
1. Molecular volume
2. Ellipsoidal volume
3. Total dipole
4. Lipophilicity (LogP)
5. Kier ChiV1
6. Kier ChiV3
7. Kier ChiV 4
8. Balaban index
9. Number of atoms
10. Number of H-bond donors
11. Number of H-bond acceptors
12. Number of H atoms
13. Number of C atoms
14. Number of N atoms
15. Number of F atoms
16. Number of S atoms
17. Group count for methyl
18. Group count for hydroxyl
19. Group count for methoxyl
20. Group count for carboxyl
21. Group count for acetamide
22. Group count for aminium

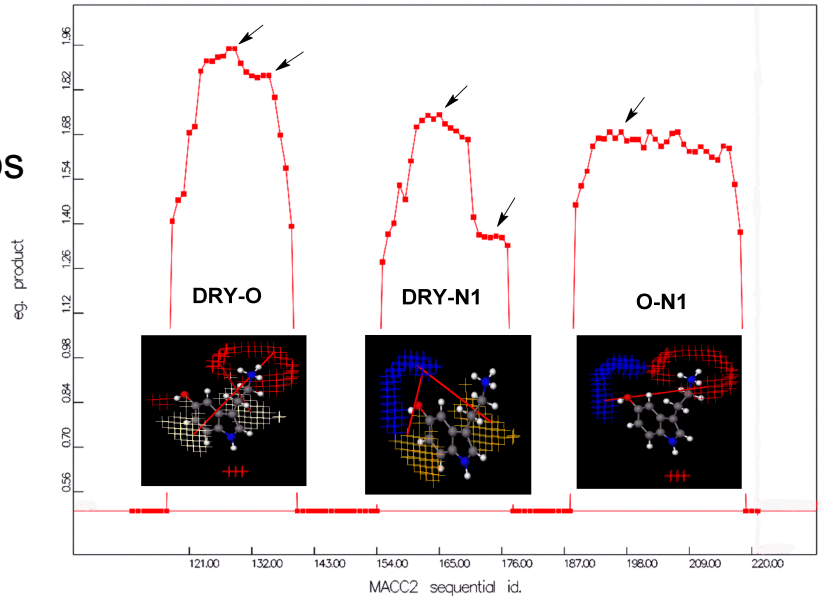
3D-QSAR

Calculation of 3D-descriptors

- **DRY probe** → hydrophobic interactions
- **O probe** (carbonyl oxygen) → H-bond acceptor groups
- **N1 probe** (amide nitrogen) → H-bond donor groups

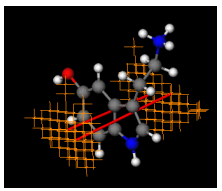


GRIND descriptors

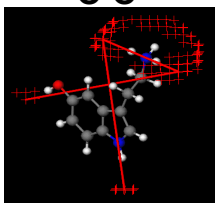


	Probe 1	Probe 2	Interaction between nodes of type:
Auto-correlograms	DRY	DRY	Hydrophobic
	O	O	H-bond donor
	N1	N1	H-bond acceptor
Cross-correlograms	DRY	O	hydrophobic and H-bond donor
	DRY	N1	hydrophobic and H-bond acceptor
	O	N1	H-bond donor and H-bond acceptor

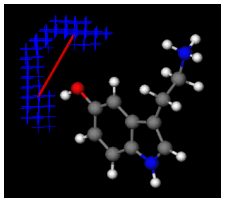
DRY-DRY

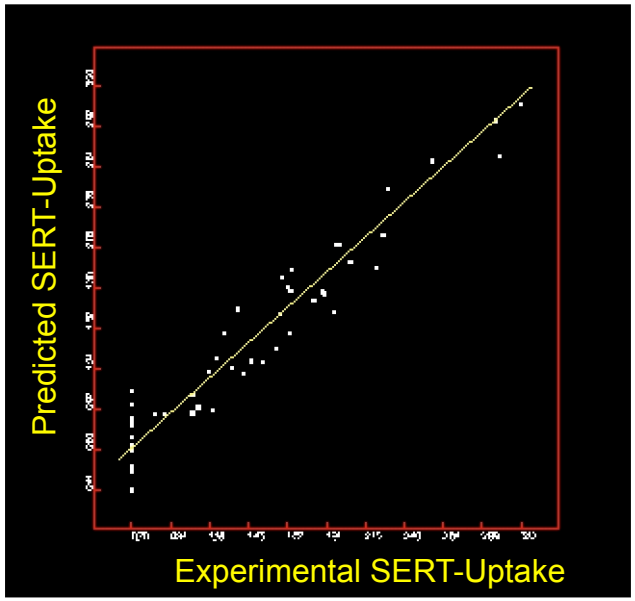


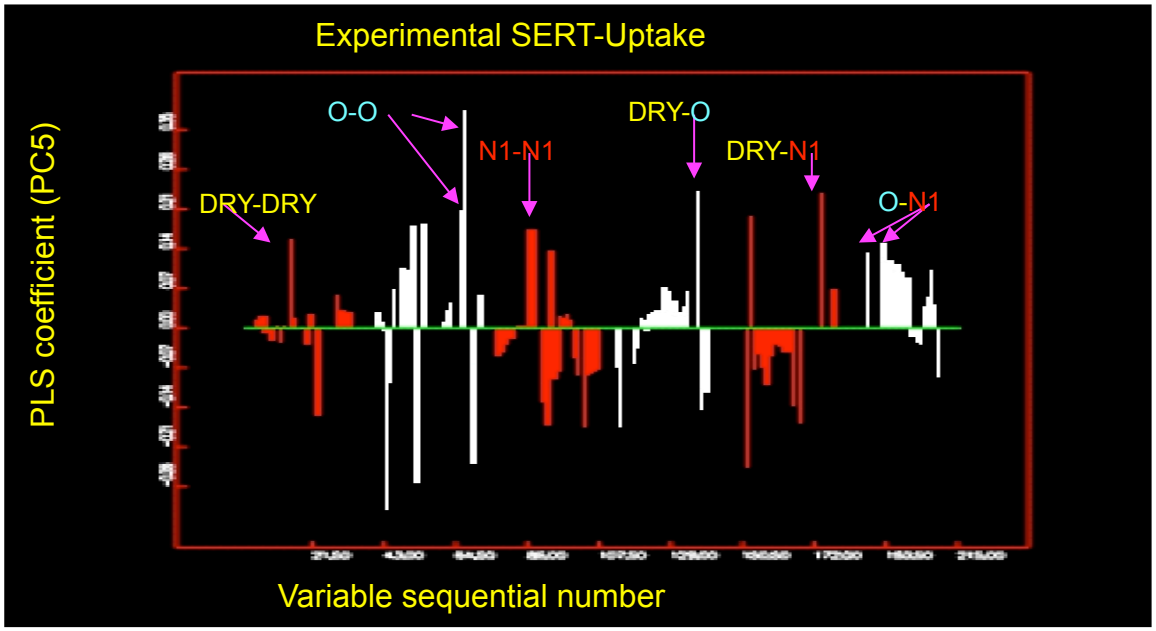
O-O

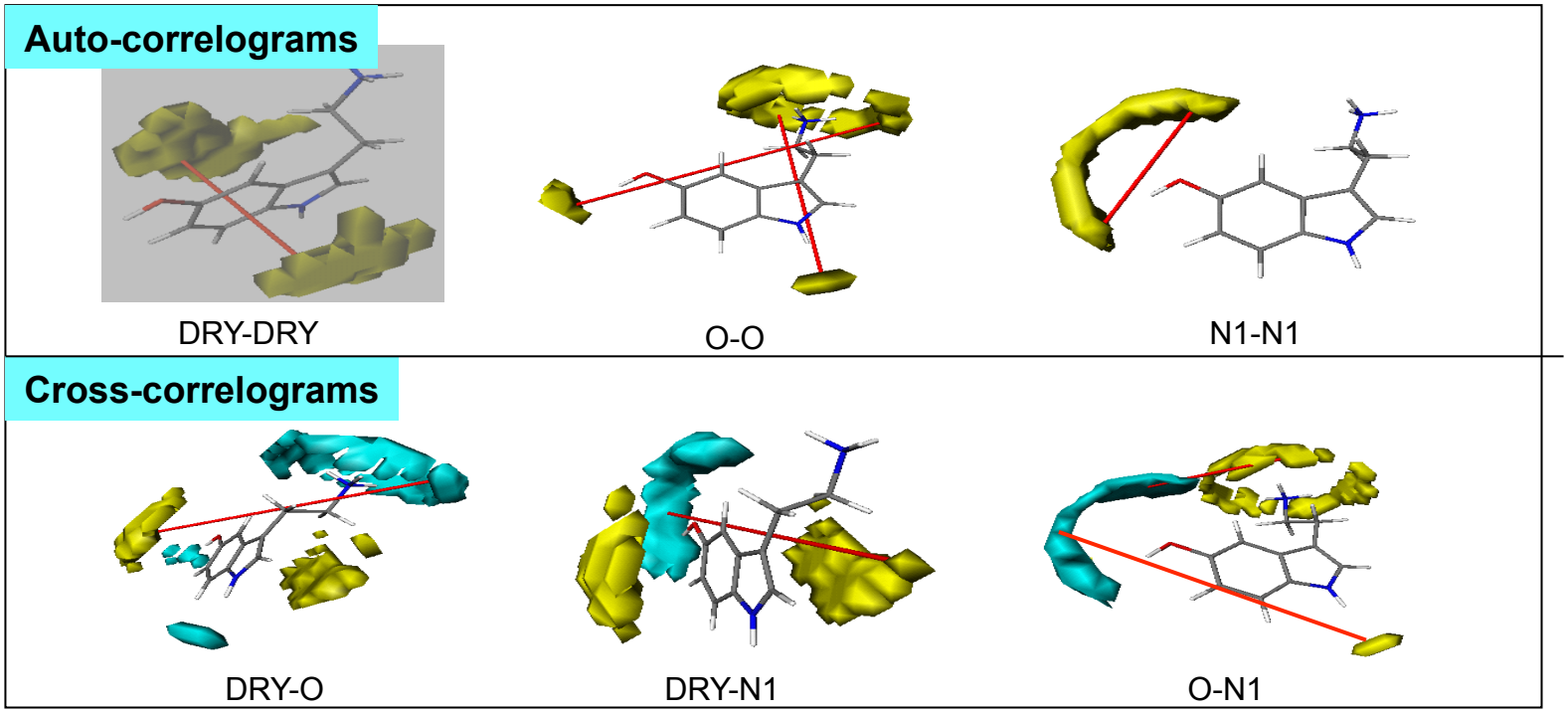


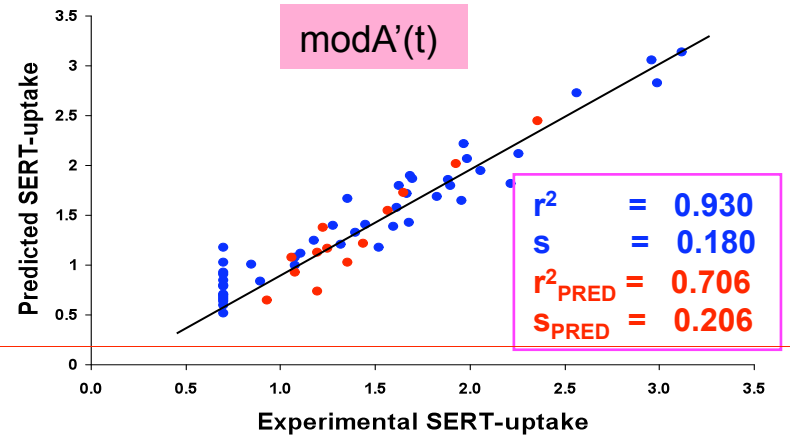
N1-N1

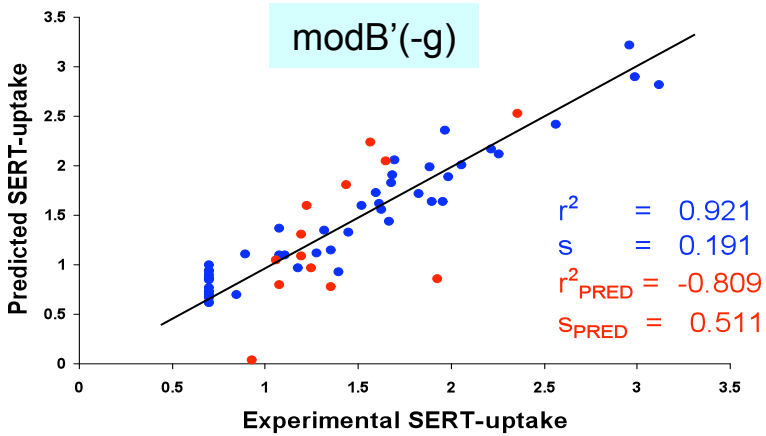


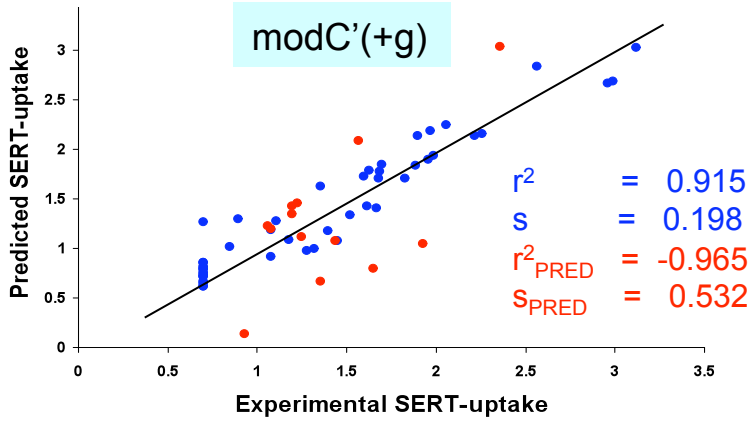

Predicted SERT-Uptake
Experimental SERT-Uptake


Experimental SERT-Uptake
PLS coefficient (PC5)
O-O
N1-N1
DRY-O
DRY-N1
DRY-DRY
O-N1
Variable sequential number

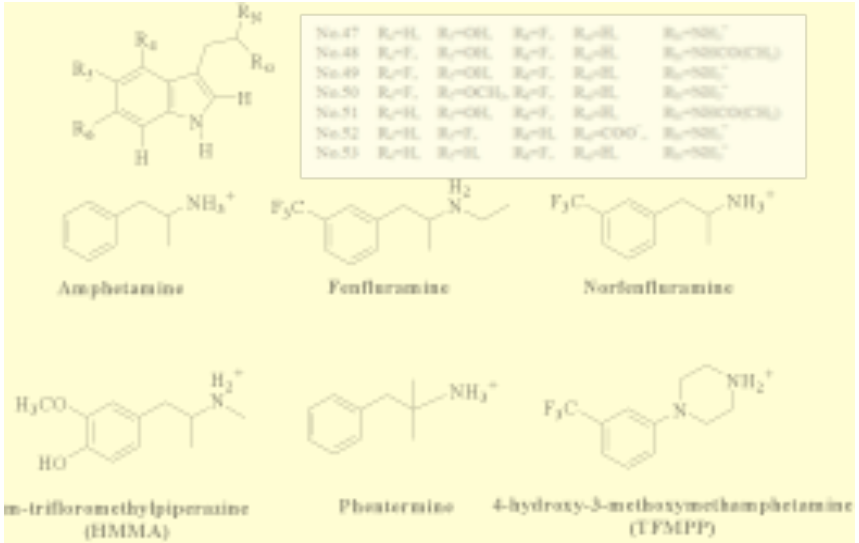

Auto-correlograms
DRY-DRY
O-O
N1-N1
Cross-correlograms
DRY-O
DRY-N1
O-N1



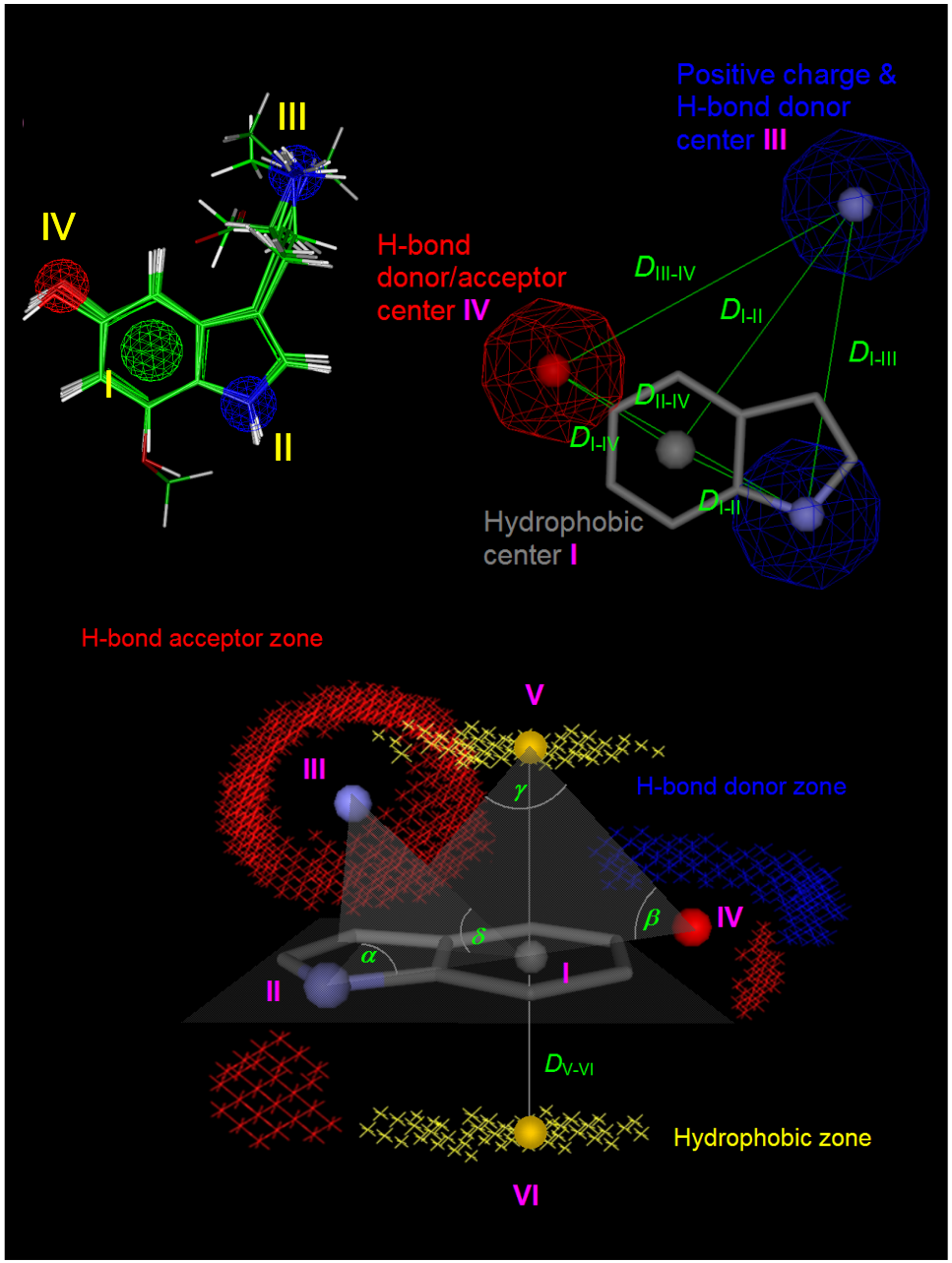




Statistical parameters	Models					
	without FFD			with FFD		
	modA (*t*)	modB (-*g*)	modC (+*g*)	modA' (*t*)	modB' (-*g*)	modC' (+*g*)
No.of descriptors	185	177	169	133	135	124
No.of components	5	6	5	5	6	5
q^2_{LOO} [a]	0.600	0.447	0.531	0.733	0.695	0.752
s_{LOO} [b]	0.428	0.504	0.464	0.350	0.374	0.337
q^2_{LFO} [c]	0.554	0.401	0.460	0.684	0.650	0.694
s_{LFO} [d]	0.452	0.524	0.498	0.381	0.401	0.375
r^2 [e]	0.911	0.906	0.894	0.930	0.921	0.915
s [f]	0.203	0.207	0.220	0.180	0.191	0.198
r^2_{PRED} [g]	-	-	-	0.706	-0.809	-0.965
s_{PRED} [h]	-	-	-	0.206	0.511	0.532

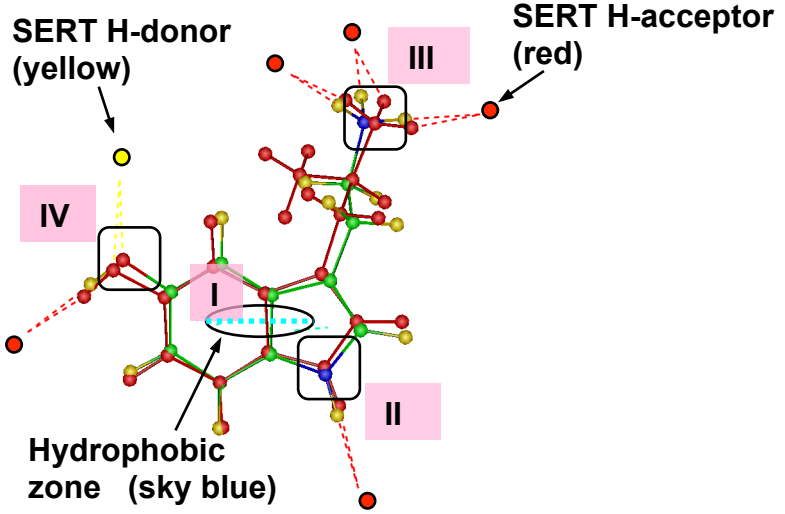


SERT-uptake Pharmacophore

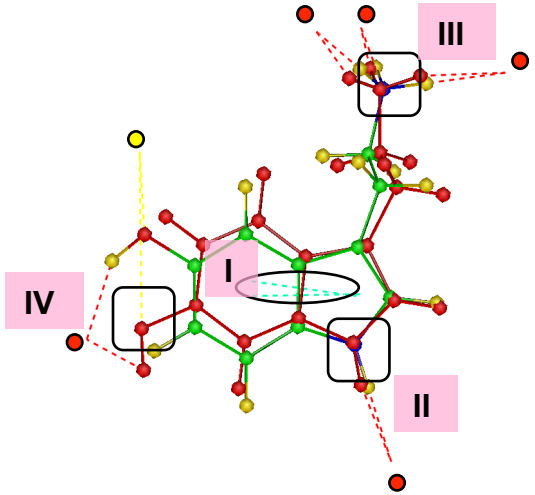


pharmacophoric components	mean ± SD (unit)
Distance	***(Å)***
D_{I-II}	2.75 ± 0.02
D_{I-III}	5.78 ± 0.11
D_{I-IV}	2.77 ± 0.02
D_{II-III}	5.89 ± 0.06
D_{II-IV}	5.51 ± 0.01
D_{III-IV}	6.60 ± 0.26
D_{I-V}	3.29 ± 0.20
D_{I-VI}	3.40 ± 0.20
D_{V-VI}	6.54 ± 0.20
Angle	***(°)***
α	57.39 ± 2.23
β	70.70 ± 3.59
γ	51.91 ± 1.44
δ	14.83 ± 1.32

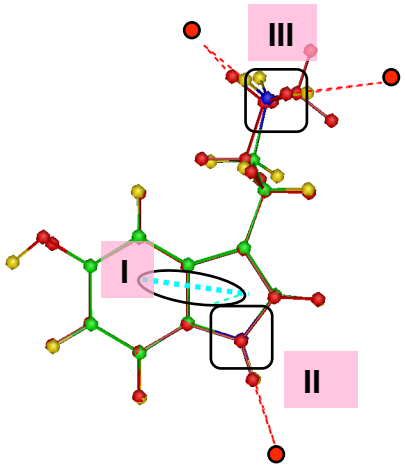
I = hydrophobic interaction and π-electron delocalisation centre of aromatic indole ring
II = hydrogen bond donor centre within indole ring
III = hydrogen bond donor and/or electrostatic interaction centre of cationic head
IV = hydrogen bond donor and/or acceptor centre located on 5-hydroxyl group
V &VI = centre of hydrophobic field localised upper and lower of indole ring



Compound 2
(9.87 ± 0.12)



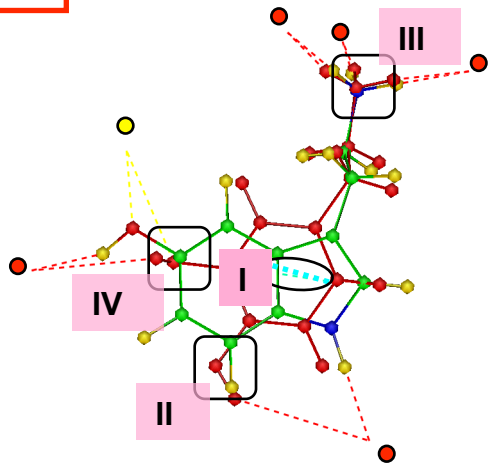
Compound 15
(0.49 ± 0.10)



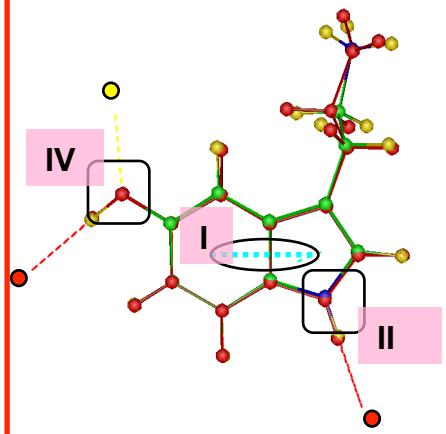
Compound 24
(0.23 ± 0.02)

The importance:

(i) the number of fitting pharmacophoric points,
(ii) the geometrical constraints (distances and angles) and
(iii) the spatial distribution of virtual interacting points within the transporter,



HMMA
(0.45±0.05)



Compound 44
(< 0.05)

Summary

- **A library of 121 compounds**
  5-HT analogs, harmanes, benzothiazoles, indanones, amphetamine derivatives and substrate-type 5-HT releasers

- **SERT-uptake activities** Human blood platelets

- **Three-dimensional QSAR (3D-QSAR) of 46 compounds**
  High confident 3D-QSAR model for SERT transport activity
  non-cross-validation $r^2 = 0.930$,
  internal cross validation $q^2_{LOO} = 0.733$, $q^2_{LFO} = 0.684$,
  external test set $r^2_{PRED} = 0.706$

- **Definition of Pharmacophore of SERT-uptake.**
  A reliable 6-points pharmacophore representative of SERT-uptake activity

Definition of an uptake pharmacophore of the serotonin transporter through 3D-QSAR Analysis
(Published in *Current Medicinal Chemistry, 2005, 12, 2393-2410*)