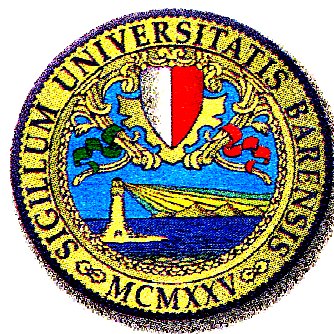


The Role of Structural Biology and Chemioinformatics in the Discovery of New Clinical Candidates for Neurodegeneration.

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<http://www.farmchim.uniba.it/mdmlab>



Barcelona, June 26, 2007

The nth shift in the Drug Discovery paradigm?

Key Players in "modern DD

(Post)-genomics <> System Biology <> Chem/Bio-informatics

New Shift in DD paradigm

One-drug > one-target  One-drug > more-targets

uni-selective  multi-selective

monopharmacology  polipharmacology

ODMT: *an approach particularly promising for multifactorial diseases, like ND diseases*

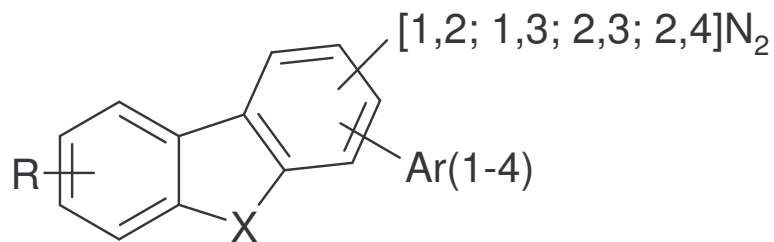
Within this new and challenging scenario

- Monoamino oxidase was selected as an appropriate target for ODMT aiming at:
- Discovery of new classes of potent, truly reversible and highly selective inhibitors
- Discovery of highly *in vivo* active inhibitors
- Discovery of dual MAO-B/AChE inhibitors
- Discovery of multitarget (multipotent) compounds with MAO-AChE inhibitory activity and additional properties eagerly pursued for the therapy of ND disease (AInf, AO,RaScav...)

Monoamine oxidases (MAOs)

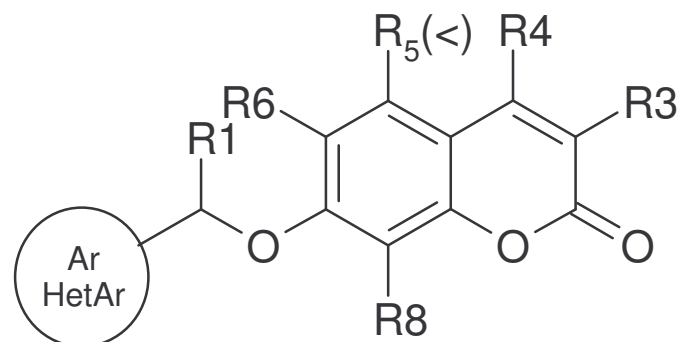
- **FAD-dependent enzymes**, involved in the oxidative deamination of endogenous and exogenous amines
- Exist as **two distinct enzymatic isoforms**, MAO-A and MAO-B, which differ in the aa sequences, 3D structures, substrate specificity, sensitivity to inhibitors and tissue distribution
- Selective MAO-A and MAO-B inhibitors are currently used as antidepressants (i.e., *moclobemide*) and as adjuvants in the L-DOPA therapy of PD (i.e. *selegiline* and *rasagiline*), respectively.
- A renewed interest on MAOs stems from the possibility to confer to selective **MAOB-Is** additional pharmacological activities (**I-AChE, AO, α A β**) highly desirable in the therapy of NDs (**multiple targets**)

Targeted structures as new MAO-Is

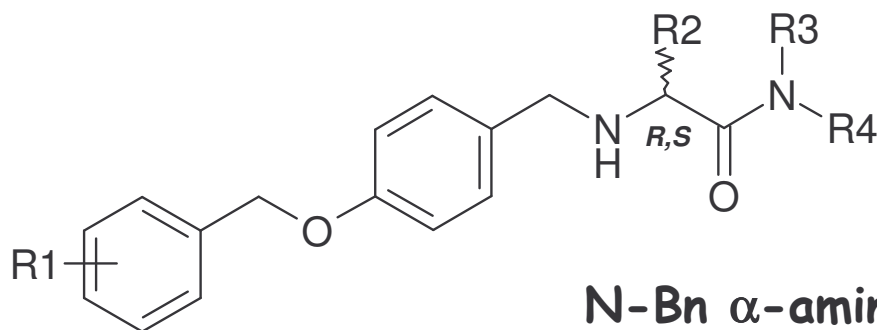


X = NH, CH₂, CO

Ind(olo)eno-diazines



Coumarins

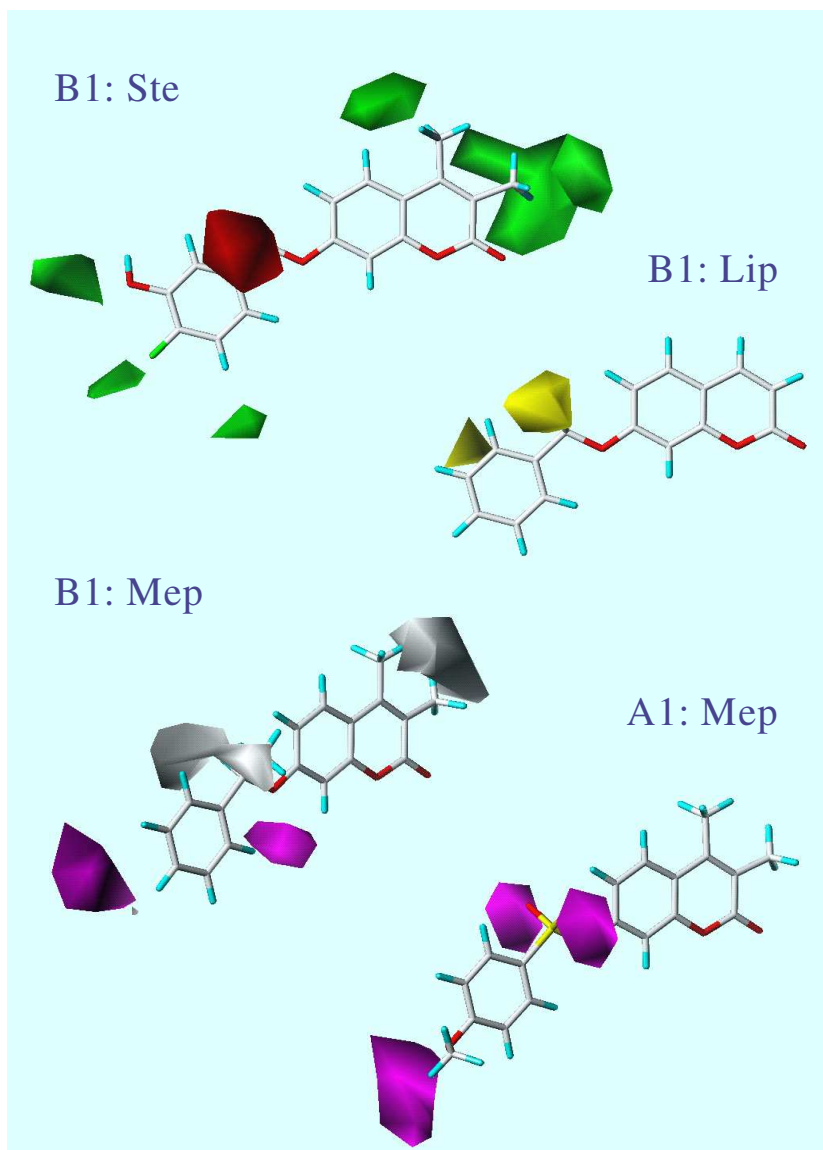


N-Bn α -aminoamides

R₁ = 3-F, R₂ = CH₃, R₃ = R₄ = H: Safinamide (Phase III)

Ligand-based studies, 2D and 3 DQSAR, began in 1999

3D QSAR-CoMFA models of Coumarins



Green: sterically allowed, favorable zones

Red: sterically forbidden, unfavorable zones

Yellow: favorable lipophilic interactions

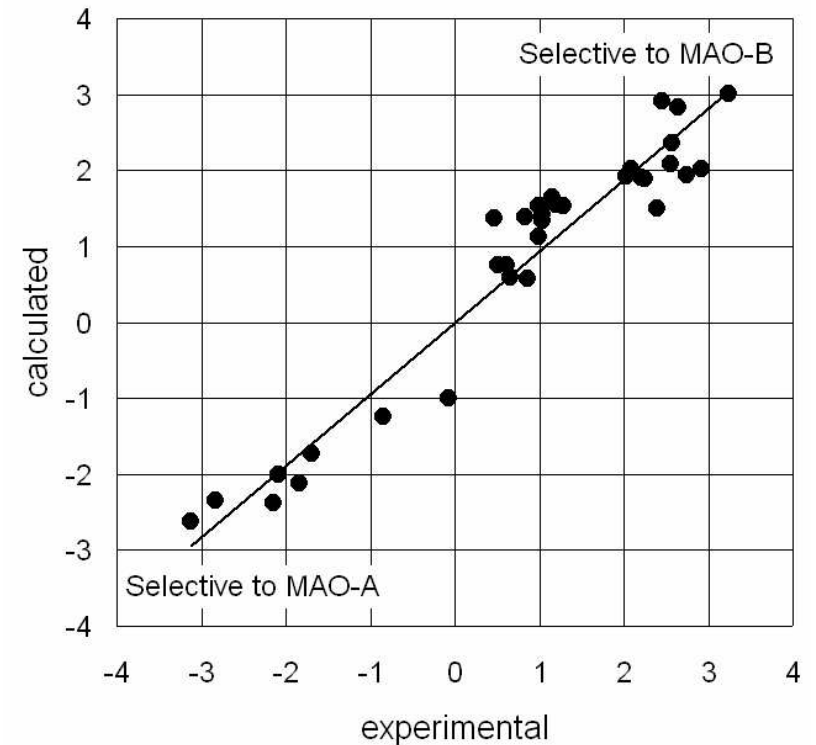
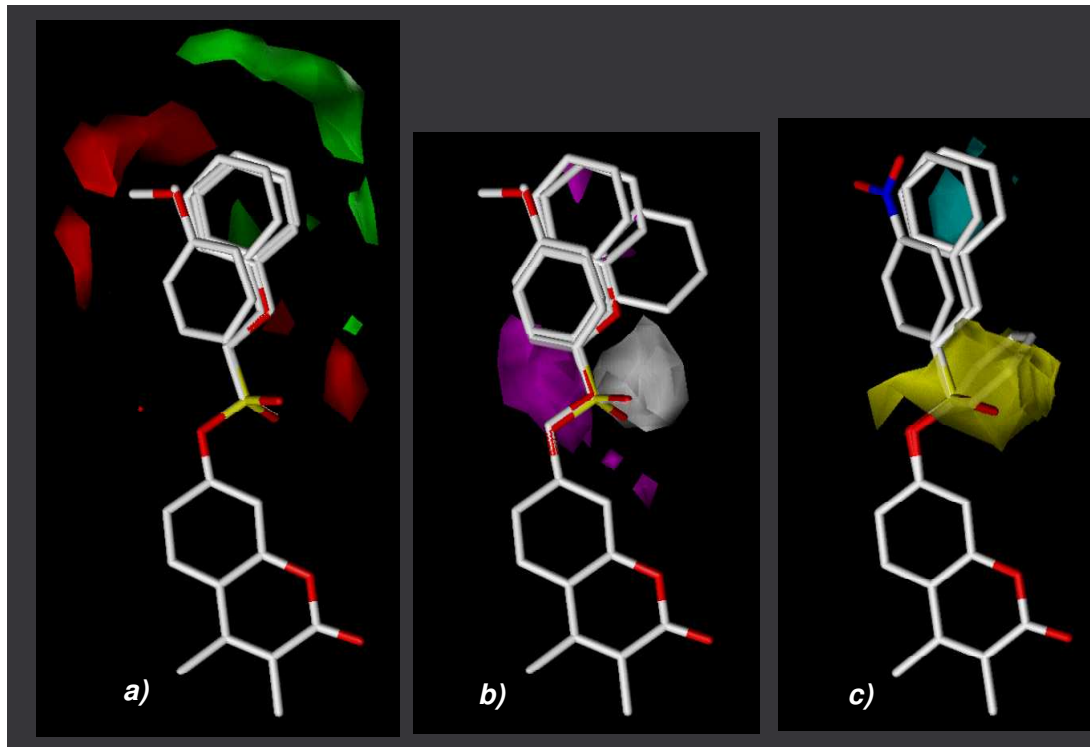
Magenta: favorable for high electron density

Gray: unfavourable for high electron density

A. Carotti & coll *J. Med. Chem.* 2000, 43, 4747

CoMFA/Golpe of B/A selectivity

a) steric, b) electrostatic and c) lipophilic isocontour maps

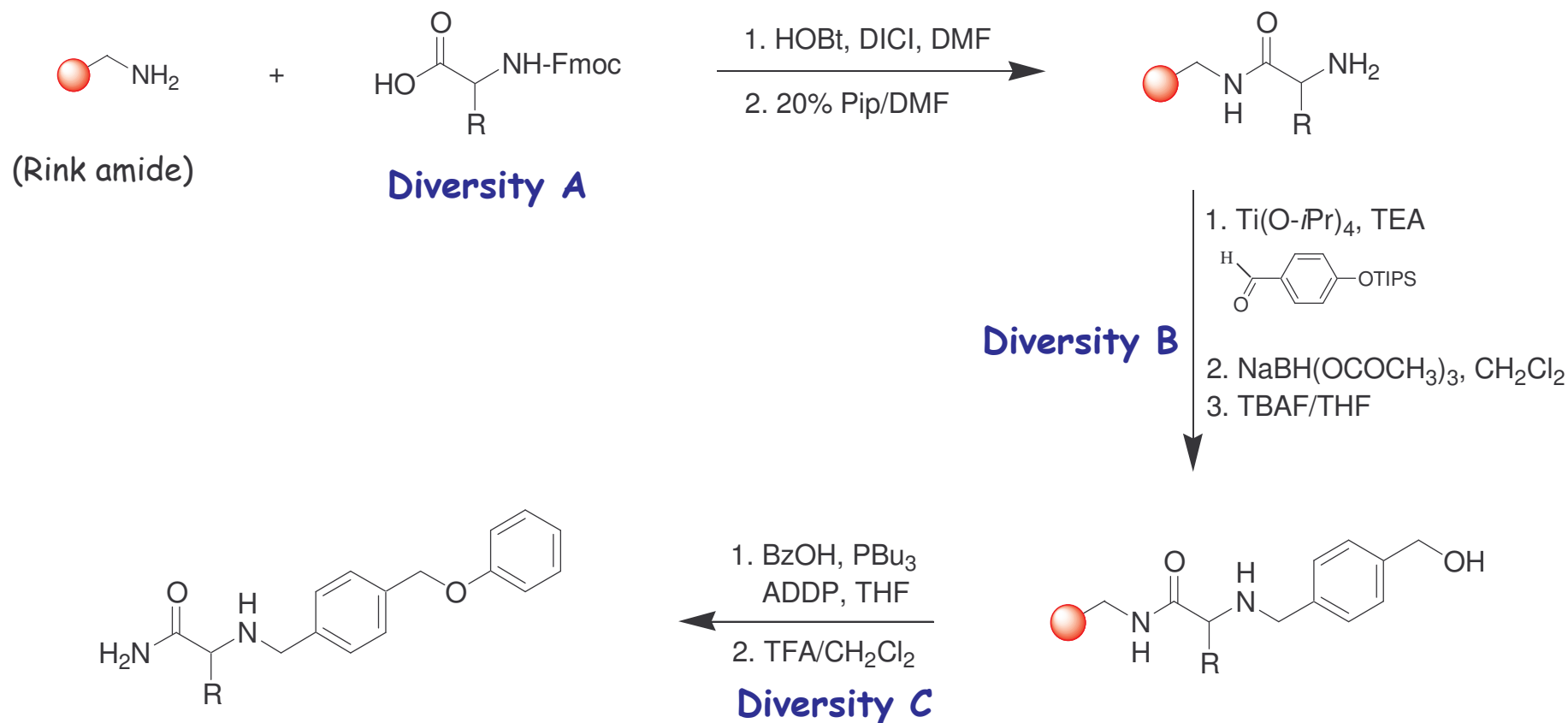


$n=34$, $onc=2$, $q^2=0.913$; $r^2= 0.940$; $SDEP= 0.506$

A. Carotti and coll *J. Med Chem.* 2006, 49, 4912

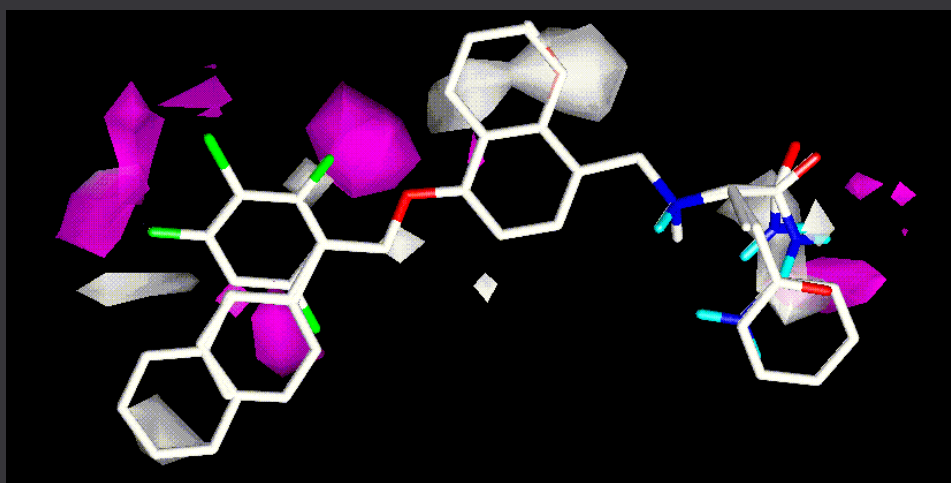
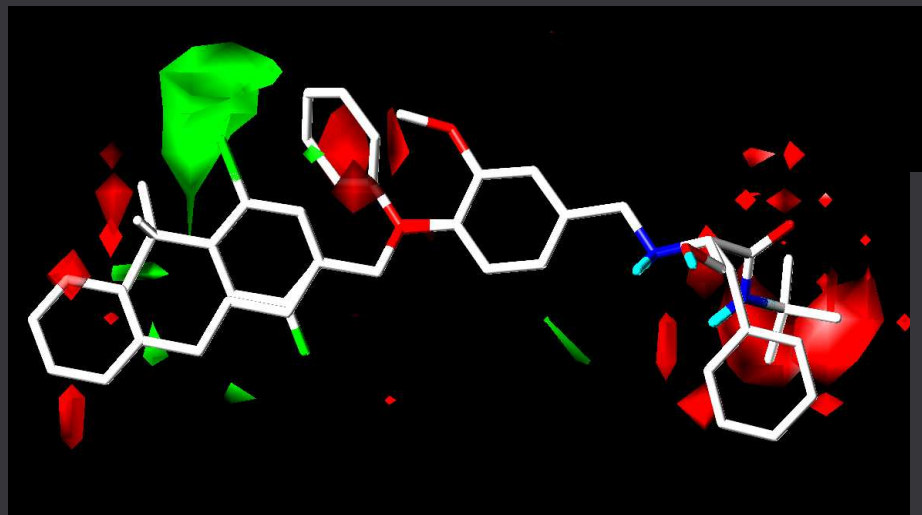
...moving to SPS of Safinamides

Solid phase synthesis of libraries of Saffinamide analogs

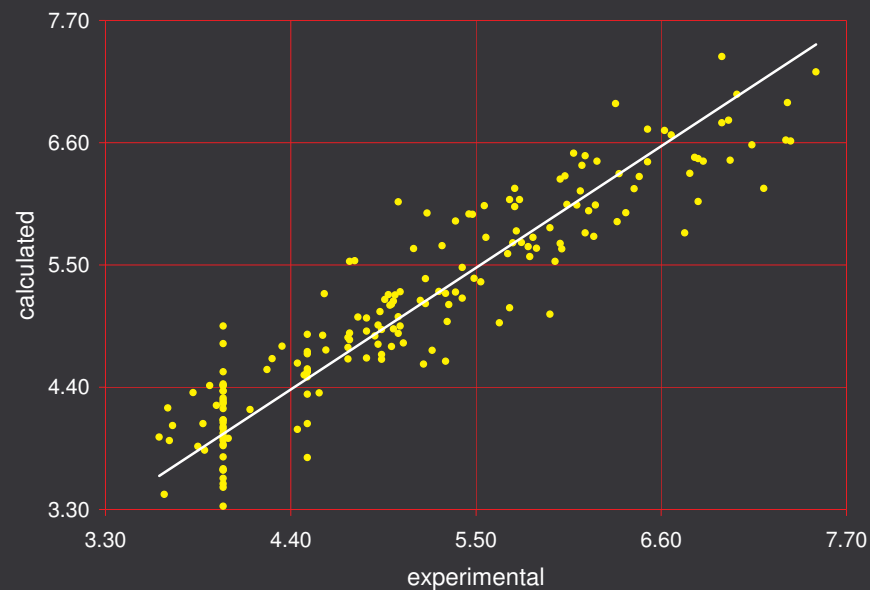


A. Carotti & coll: *J.Med.Chem*, *in press*

SAFs: CoMFA/Golpe models: statistics & contour maps



Training set



FIELD	n	ONC/q ²	r ²	SDEP
SteEle_FFD2	179	5/0.705	0.864	0.541

Prediction set

FIELD	n	ONC/r ² _{ext}
SteEle_FFD2	19	5/0.683

Moving towards direct modelling studies

Major breakthroughs in the structural and functional characterization of MAOs

"Structure of **human MAO- B**, a drug target for the treatment of neurological disorders"

C. Binda, P. Newton-Winson, F. Hubalek, D.E. Edmondson, A. Mattevi *Nat.Struct. Biol* **2002**, *9*, 1

"Insights into the mode of inhibition of **human mitochondrial monoamine oxidase B** from high-resolution crystal structures"

C. Binda, M. Li, F. Hubalek, N. Restelli, D. E. Edmondson and A. Mattevi, *Proc. Natl. Acad. Sci. U. S. A* **2003**, *100*, 9750

"Structure of **rat monoamine oxidase A** and its specific recognitions for substrates and inhibitors"

Ma, J.; Yoshimura, M.; Yamashita, E.; Nakagawa, A.; Ito, A.; and Tsukihara, T. *J. Mol. Biol.* **2004**, *338*, 103

"3D structure of **human monoamine oxidase A**: relation to the structures of rat MAO-A and human MAO-B

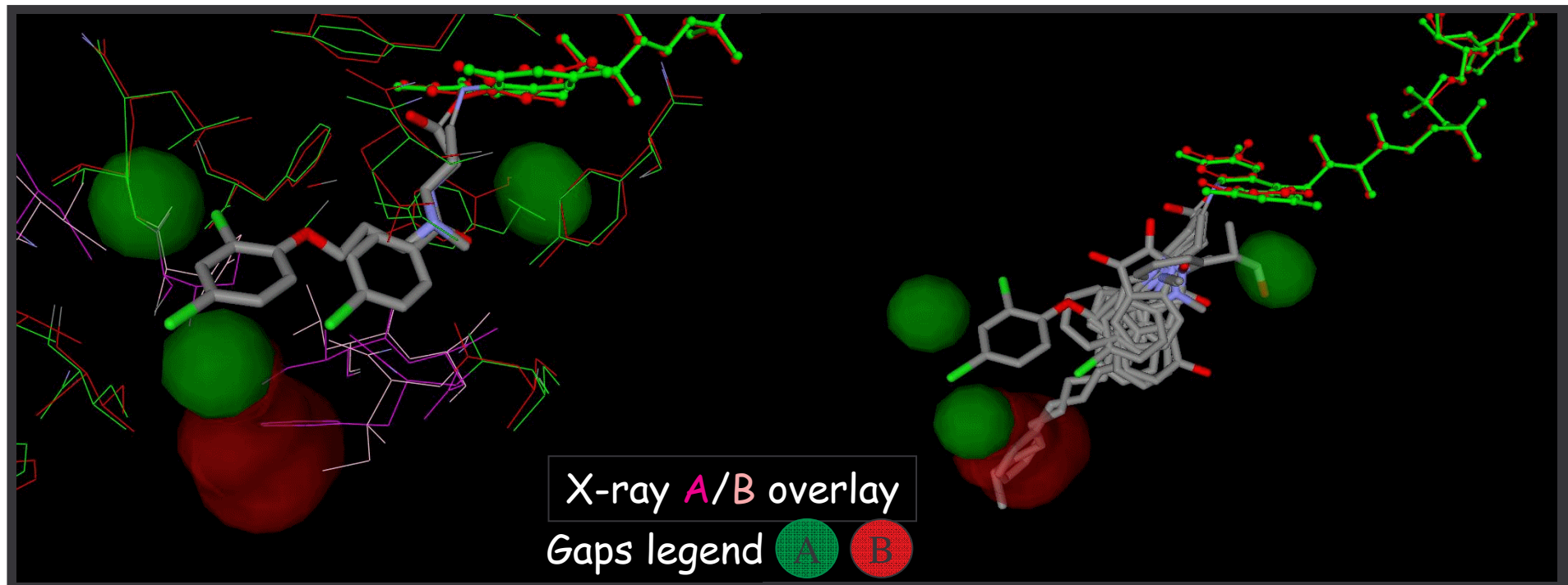
L. De Colibus, M. Li, F. Hubalek, C. Binda, A. Lustig, D. E. Edmondson and A. Mattevi, *Proc. Natl. Acad. Sci. U. S. A* **2005**, *102*, 12684

Concrete opportunity for SBD & for comparing 3D QSAR models with X-ray structure

Looking for reliable explanations of MAO-A/MAO-B selectivity

MAO-A/B cavity gap comparison (Surfnets)

Common binding mode of MAO-B inhs (X-ray)



Clorgyl. vs daLazab

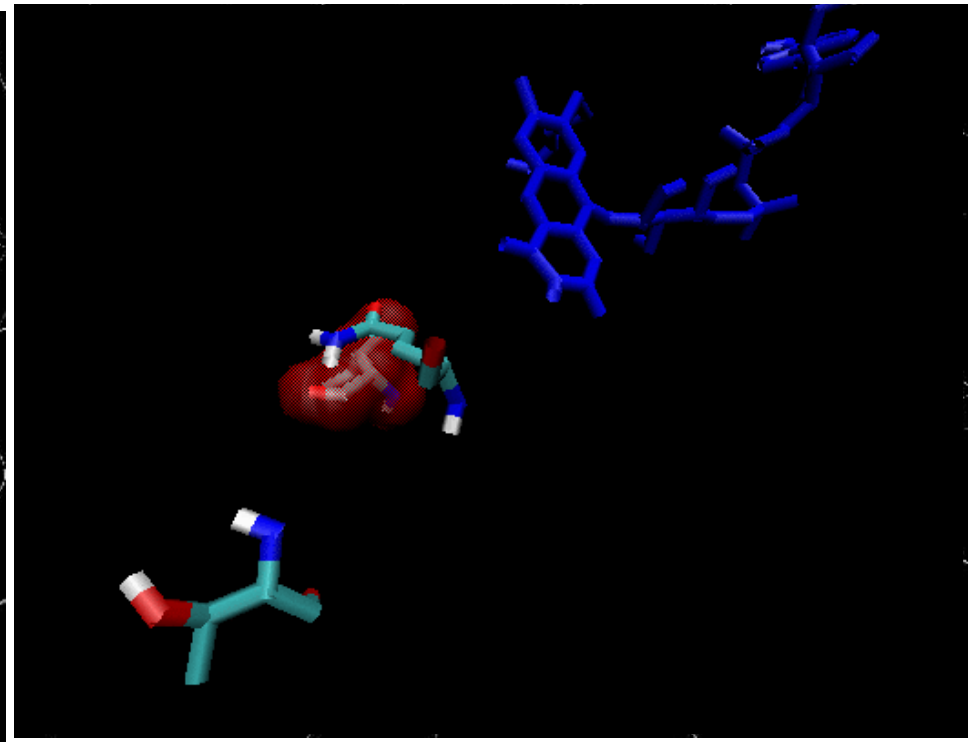
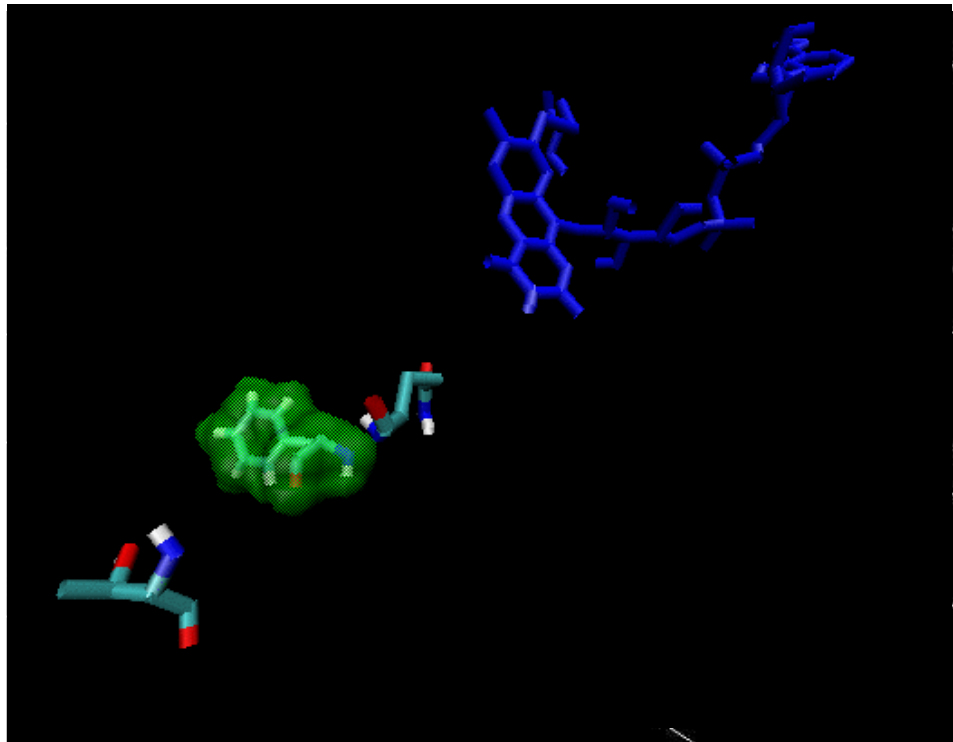
Ten covalent MAO-B inhs + Clorgyl.

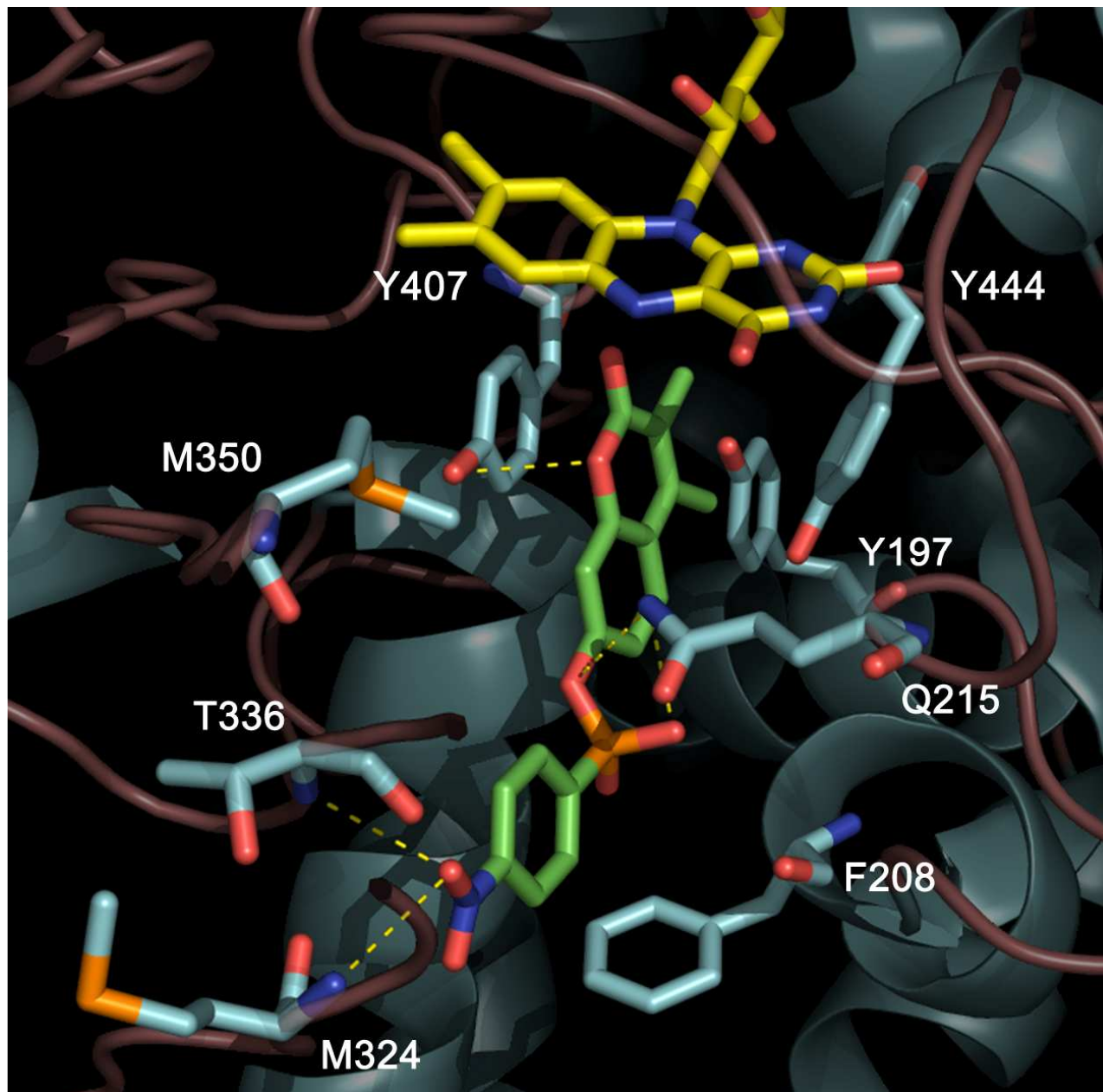
Moving to MD, looking for different aa mobilities Q215 (A) and 206(B); T211 (A) and 202(B)

Different conformational mobility of Q 215 (A) and 206 (B) and T 211 (A) and 202 (B)

MAO A

MAO B





7-coumarin derivativ.
(p.nitroPhsulphonate)
docked on rMAO-A

*(numbering refers to
rMAO-A; GOLD sw)*

Binding topology
Coum >>>> FAD

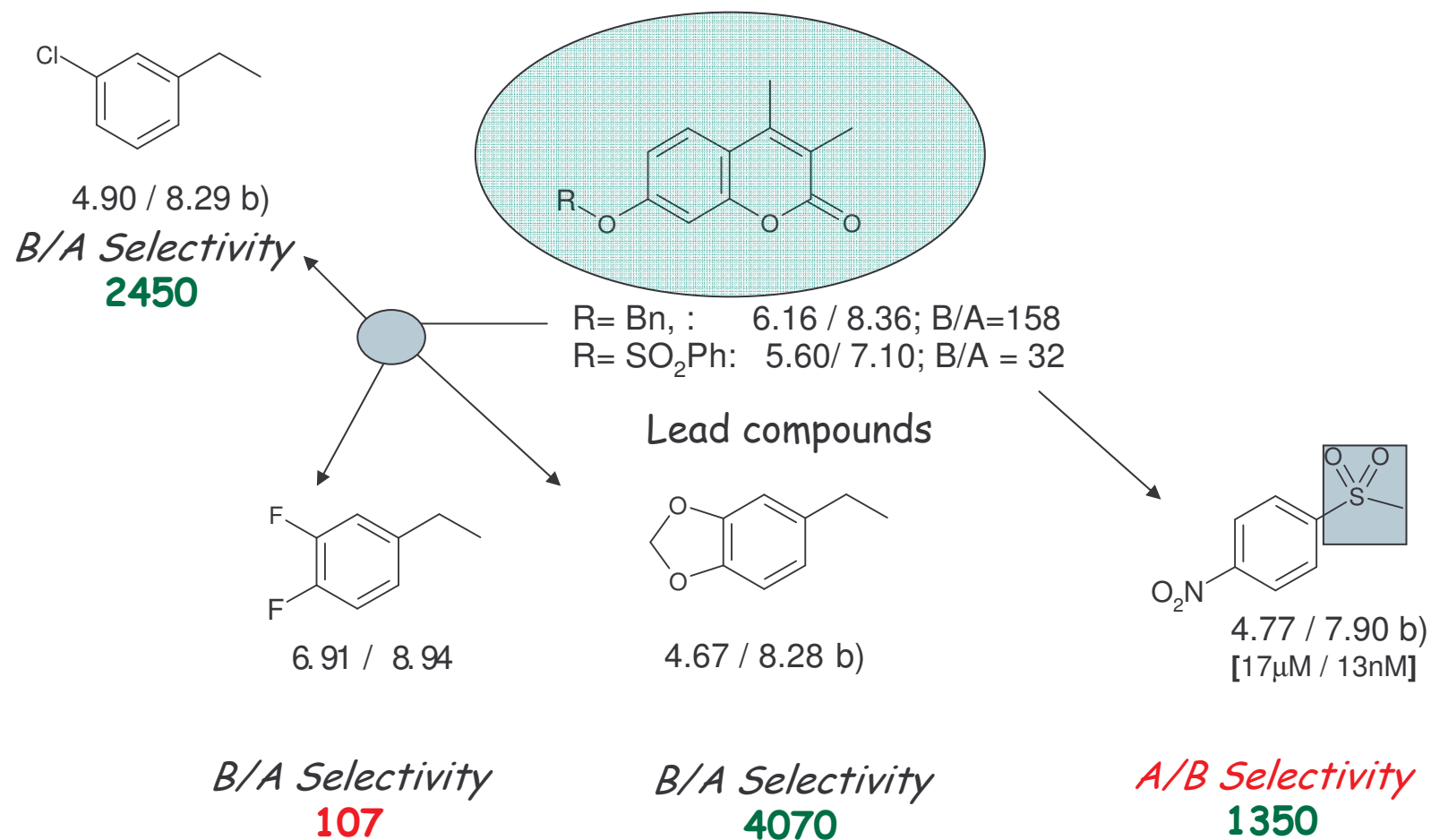
A. Carotti & coll.
J. Med. Chem.,
2006, 49, 4912



Herakleitos (535 AC - 475 AC)

*Man who desires to know the world must learn
to know it from the particulars*

Successful optimization of MAO-A and MAO-B affinities and selectivities



(Poor PK properties and drug-likeness)

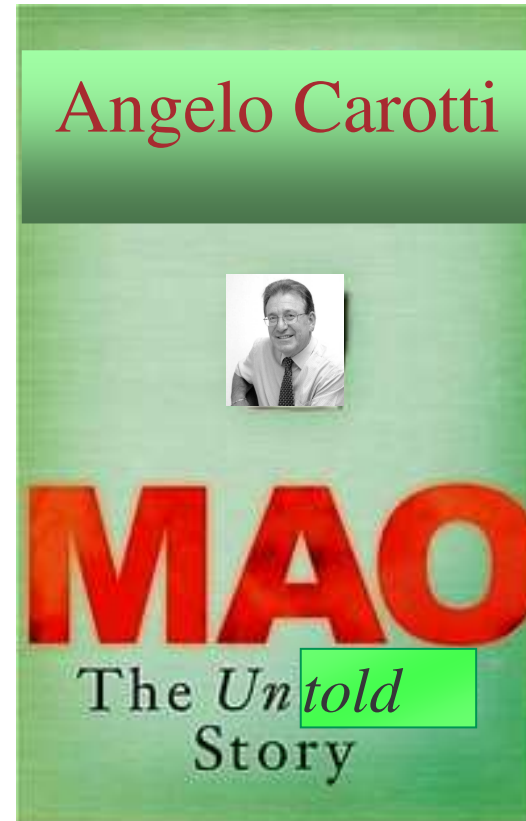
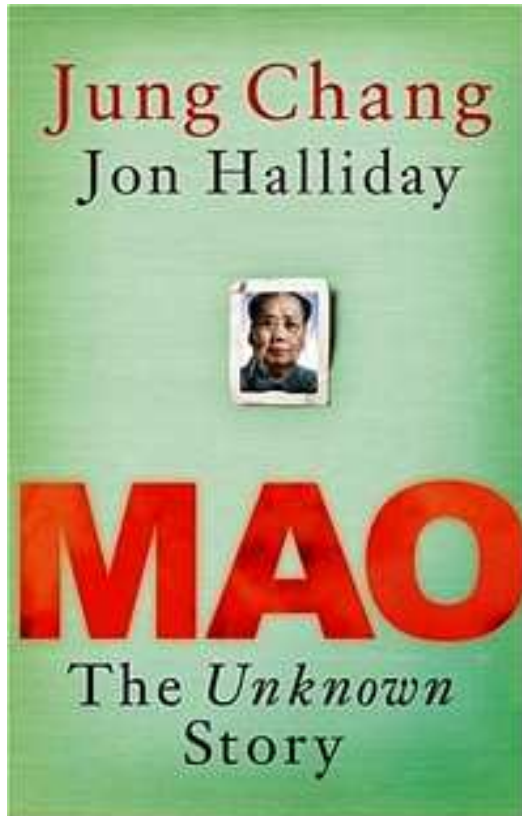
A. Carotti & coll .*J. Med. Chem.* **2000**, *43*, 4747; *J. Med. Chem.* **2001**, *44*, 3195; *J. Comp.-Aid Mol. Des.* **2002** ,*16*, 769; a) *Chem. & Biodiv.* **2006**, *3*, 134 and b) *Unpublished results*

Design of a new class of potent, selective and *in vivo* active, MAO-B and MAO-A inhibitors

MAO-A inhibitors : IC_{50} 1-10 nM; A/B selectivity > 5000

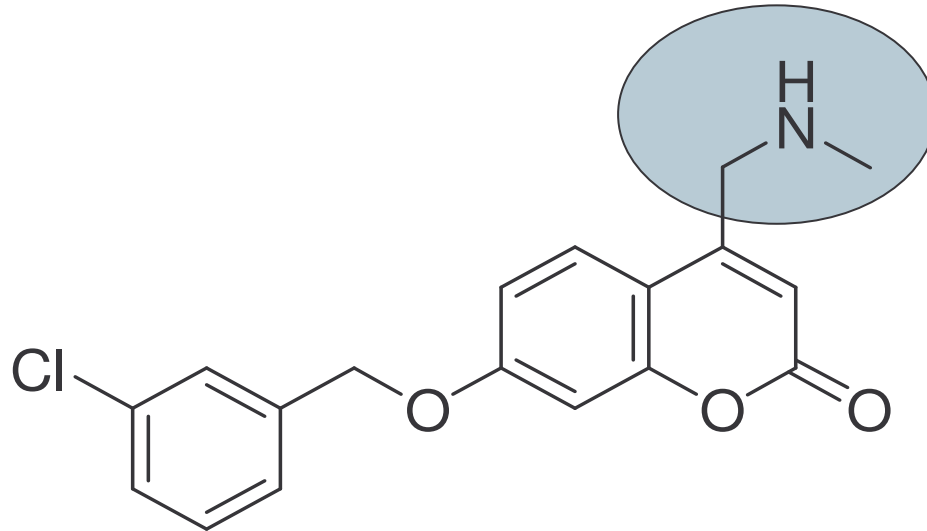
MAO-B inhibitors : IC_{50} 5-20 nM ; B/A selectivity > 600

- *Easy synthetic accessibility*
- *High "drug-likeness"*



EFMC Meeting , Vienna June 21, 2005.

The disclosure



NW-1771 :.... new lead tows. MuPoLi

Simple structural modifications led to an outstanding improvement of the pharmacokinetic and DL properties

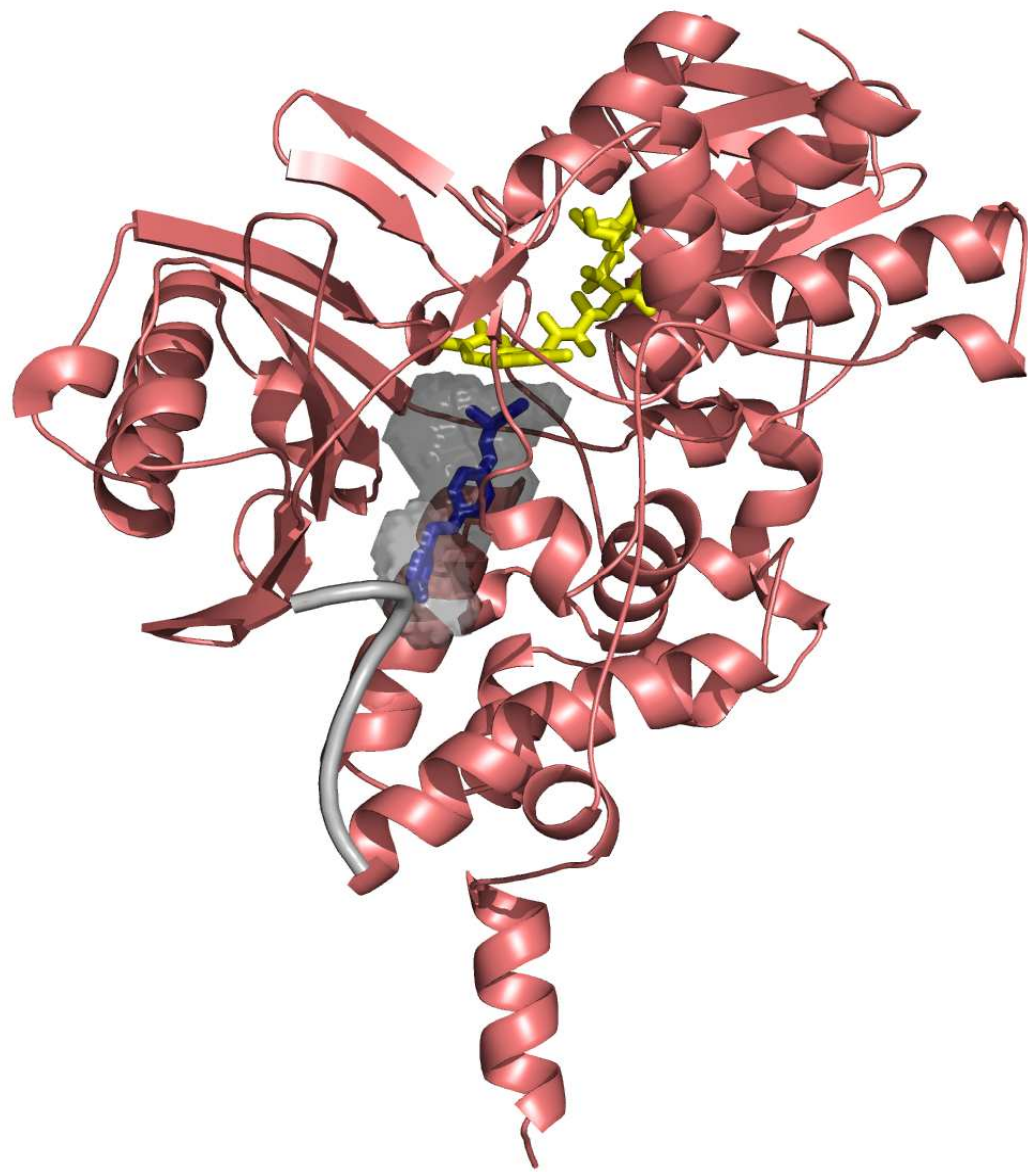
A. Carotti, P. Melloni, F. Thaler, C. Caccia, S. Maestroni, P. Salvati *WO 2006, 102958 A1*

Summary of *in vitro/in vivo* activities of NW-1771

- Highly potent MAO-B I (both in rat and human enzymes, 6-11 nM)
- Highly selective MAO-B vs MAO-A I (> 500-fold)
- Reversible MAO-B I
- Very potent MAO-B I *in vivo* (i.p., os, $EC_{50} = 0.5$ mg/kg)
- Short-acting MAO-B I (16 h full recovery of MAO-B activity)
- High and rapid brain penetration (0.5 h)
- Orally available
- No particular liability on selected CYP isoforms
- Non toxic

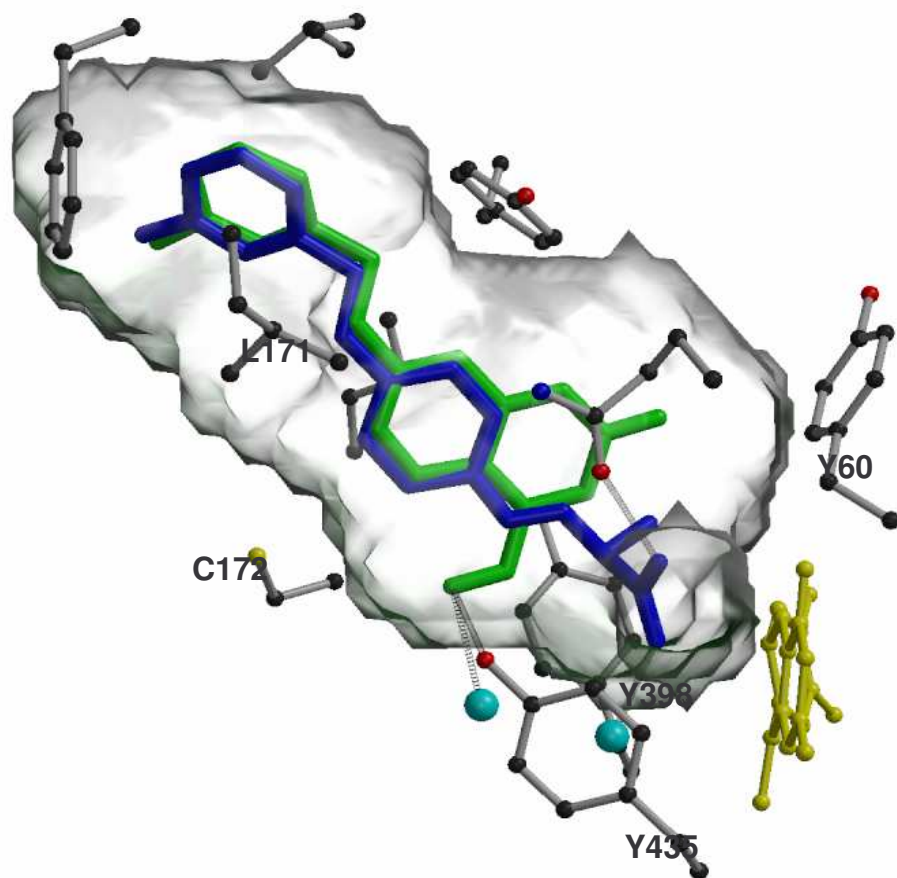
Moving towards structural biology.....

X-ray Crystal Structure of SAF with hMAO-B



Binda, Mattevi, Edmonson,
Carotti et al *J.Med.Chem* ,
in press

X-ray Crystal Structures of **SAF** and **NW-1771** complexes with hMAO-B



Binda, Mattevi, Edmonson,
Carotti et al *J.Med.Chem* ,
in press

Multi-target activities of newly designed MAO-Is

Class	MAO-A*	MAO-B*	AChE*	BChE*
IPh	50	10.1	0.84	3.9
IP 1	>100	3.2	0.055	26@10
IP 2	>100	11	0.018	20@10
COU 1	2	0.015	16	ND
COU 2	5.6	0.014	6	ND
COU 3	51	1.1	8.3	ND
COU 4	>100	9.1	0.030	ND
COU 5	>100	4.1	2.7	34@10
Sc HOP 1	>100	10	1.4	17@10

(*) IC₅₀ or % of inhibition @ μM conc.

Additional activities of some coumarin derivatives: preliminary findings

- Good antiinflammatory activity, close to that of Indomethacin (carrageenan-induced paw edema)
- Good antioxidant and radical scavenging properties (LOX, $\text{OH} \cdot$, superoxide anion)

Conclusion and prospects

- Integrated *brain tuning*, indirect and direct modelling approaches led to the discovery of new, potent MAO-B and MAO-A selective inhibitors, with potential in neurological disorders
- NW-1771 proved to be an outstanding *in vitro* and *in vivo* active and selective MAO-B inhibitor with promising drug-like properties
- Dual AChE/MAO-B inhibitors endowed with additional, anti-inflammatory, antioxidant and radical scavenging properties have been discovered.
- *In vivo* animal assays will definitely prove the validity of our multitarget approach to ND diseases, in particular AD (and PD?)

Acknowledgements

Synthesis	Modelling & Library design
Francesco Leonetti	Orazio Nicolotti
Marco Catto	Andrea Carotti
Carmelida Capaldi	Cosimo Altomare
Gianni Muncipinto	
Leonardo Pisani	All calculation were performed on a home-made linux cluster of 16 nodes (openMosix®).
Eddy Sotelo -USC (Visit. Prof)	
Angela Stefanachi	

External collaborations

- Proff. Pierre-Alain Carrupt Université de Geneve (CH)
- Prof. Ramon Sototero, USC (ES)
- Prof. Andrea Mattevi, University of Pavia (IT)
- Newron Pharmaceutical, Bresso (IT)



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