

# Identificazione di nuovi inibitori di DOT1L mediante tecniche di modellistica molecolare

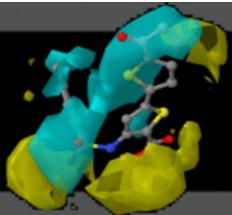


**SAPIENZA**  
UNIVERSITÀ DI ROMA

**Facoltà di Farmacia e Medicina**  
**Corso di Laurea in Chimica e Tecnologia Farmaceutiche**  
**Tesi sperimentale in Chimica Farmaceutica**  
**a.a. 2015/2016**

**Laureanda: Angelica Nigro**  
**Matricola: 1228113**

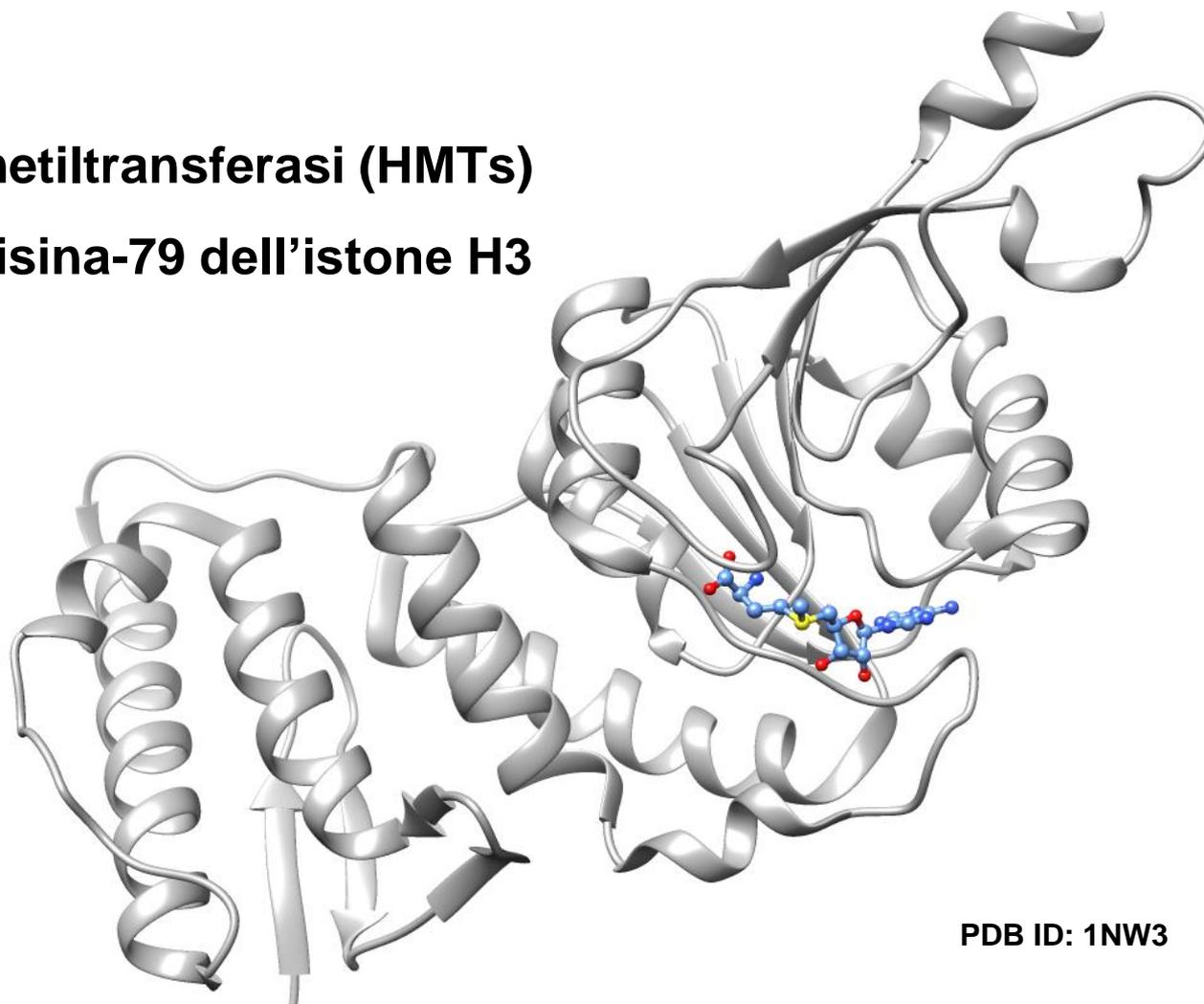
**Relatore: Prof. Antonello Mai**  
**Correlatore: Prof. Rino Ragno**



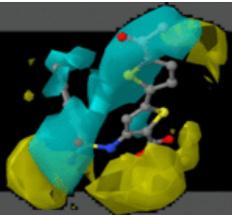
# DOT1L : Disruptor Of Telomeric Silencing 1-Like

by [www.RCMD.it](http://www.RCMD.it)

Istone-lisina N-metiltransferasi (HMTs)  
specifica per la lisina-79 dell'istone H3



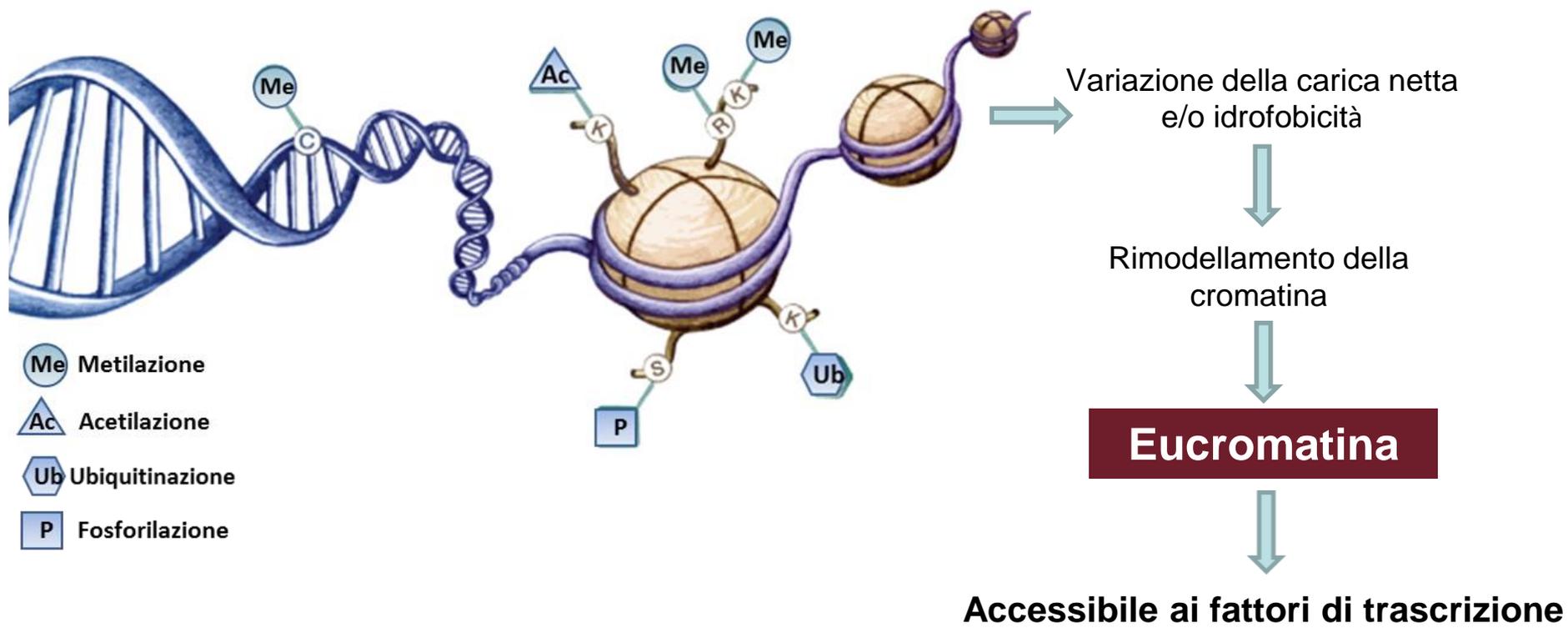
PDB ID: 1NW3

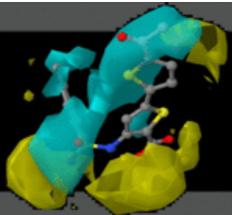


# Epigenetica

by [www.RCMD.it](http://www.RCMD.it)

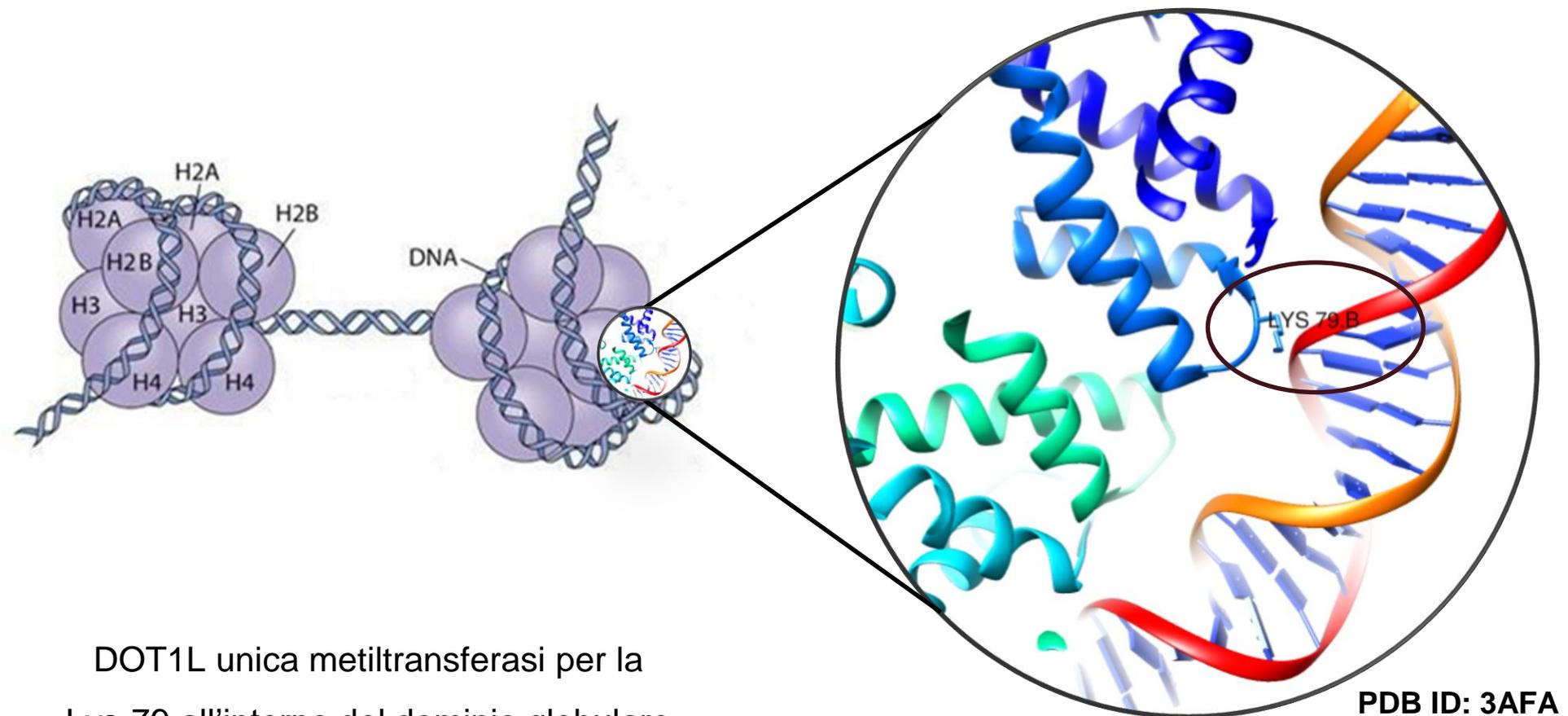
La **metilazione** delle catene  $\epsilon$ -NH<sub>2</sub> delle lisine degli istoni è una delle modifiche post-traduzionali in grado di regolare la trasmissione genica in maniera **epigenetica**.





# Nucleosoma

by [www.rcmd.it](http://www.rcmd.it)

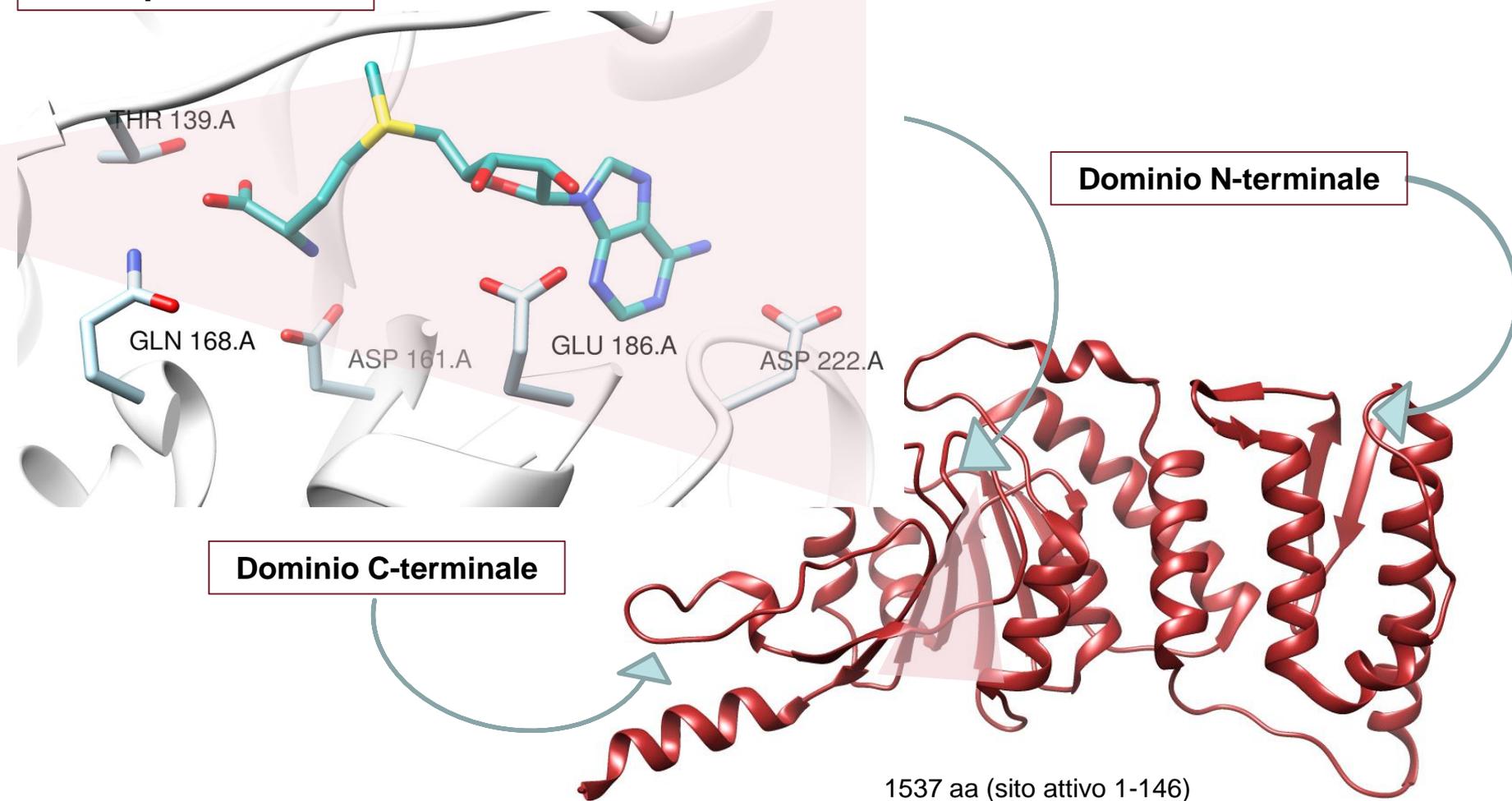


DOT1L unica metiltransferasi per la  
Lys-79 all'interno del dominio globulare  
dell'istone H3.

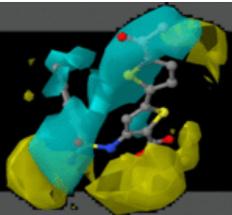
# Struttura della proteina

**Sito per la SAM**

Legami idrogeno con: Asp161, Gln168, Glu186, Thr139 e Asp222.



1537 aa (sito attivo 1-146)

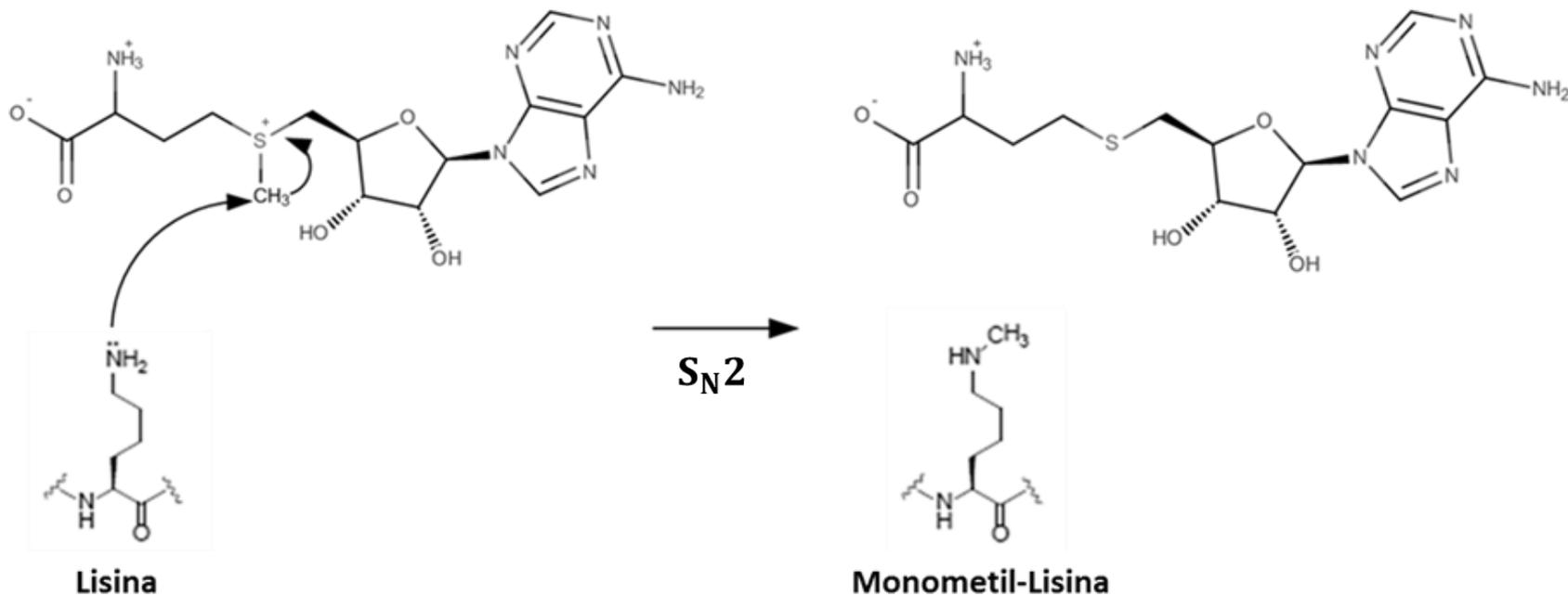


# Meccanismo metilazione della lisina *RCMD*.it

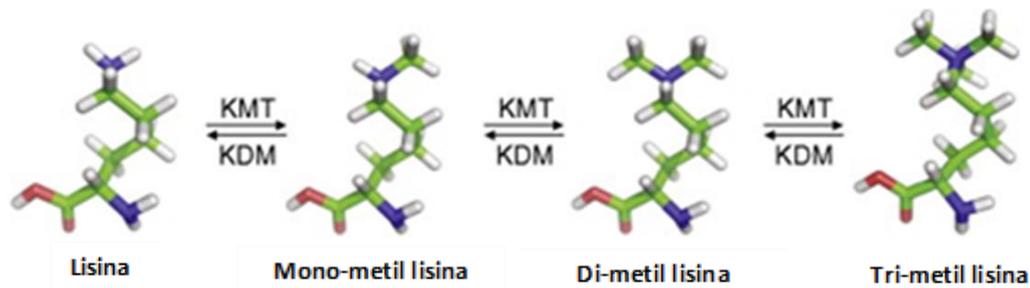
by [www.RCMD.it](http://www.RCMD.it)

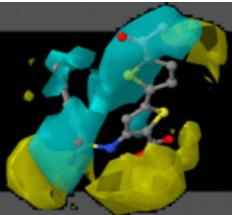
S-adenosil-L-metionina (SAM)

S-adenosil-L-omocisteina (SAH)

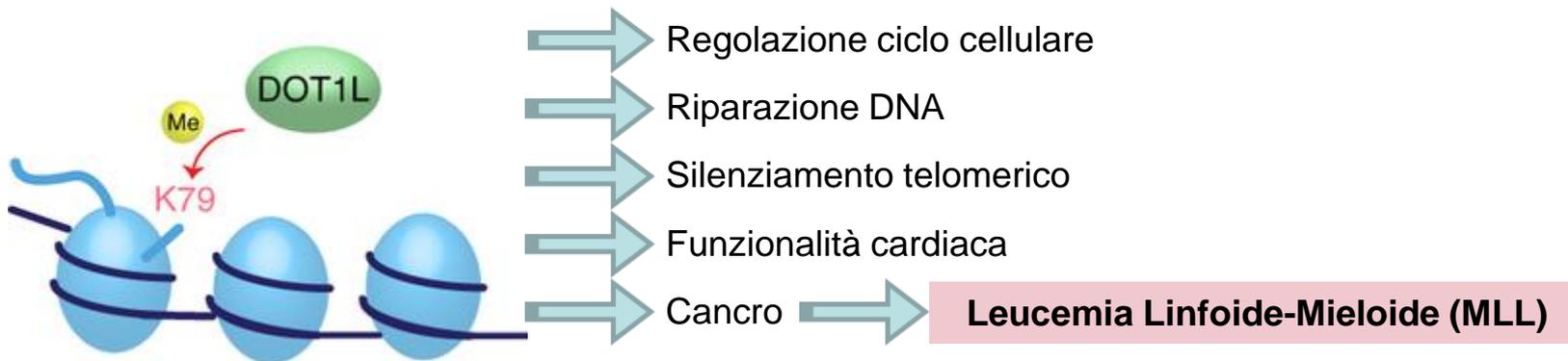


Esistono tre gradi di metilazione associati a diversi segnali per la cellula:

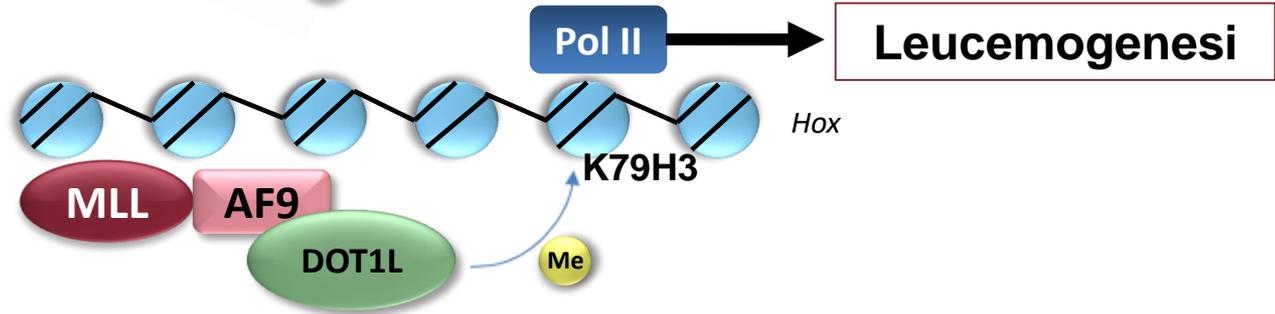
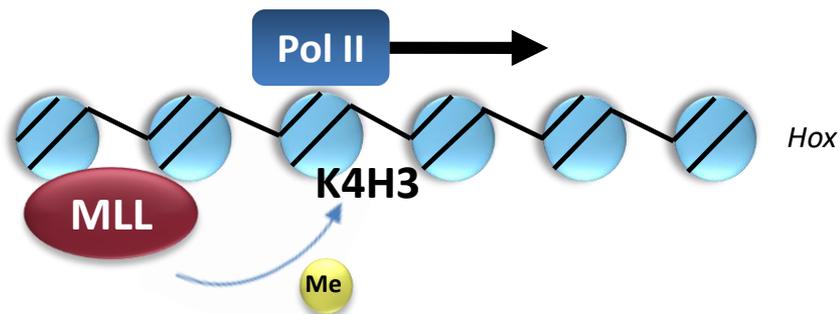


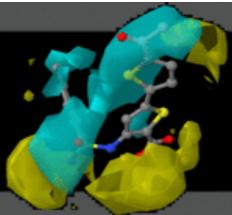


# Ruoli di DOT1L

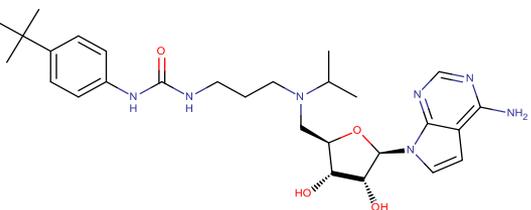


traslocazione cromosomica dal locus 11q23

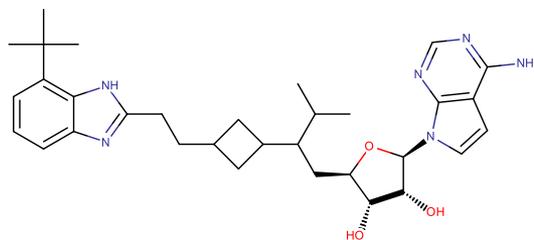




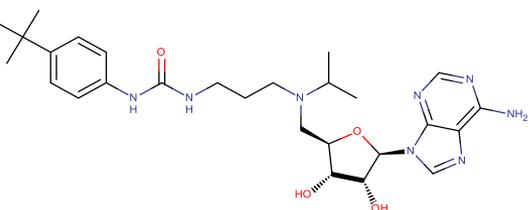
# Inibitori DOT1L



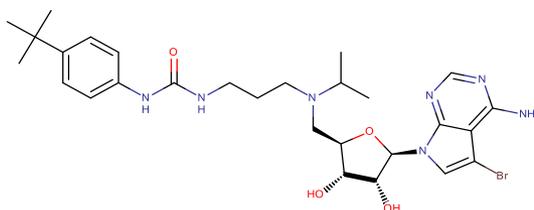
**EPZ004777** ( $K_i = 0.3 \text{ nM}$ )



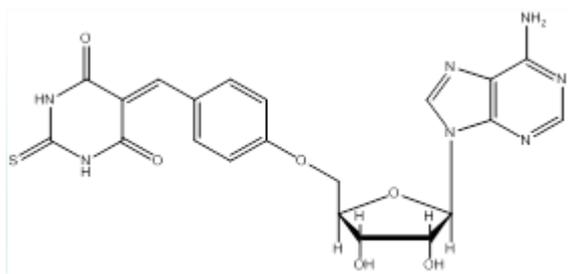
**EPZ-5676 (Pinometostat)** ( $K_i < 0.08 \text{ nM}$ )



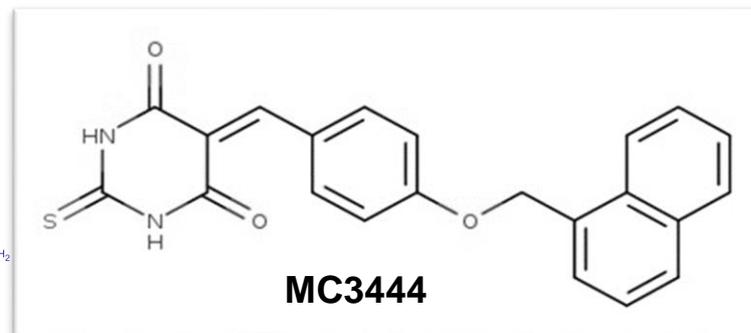
**SYC-522**



**SGC0946**

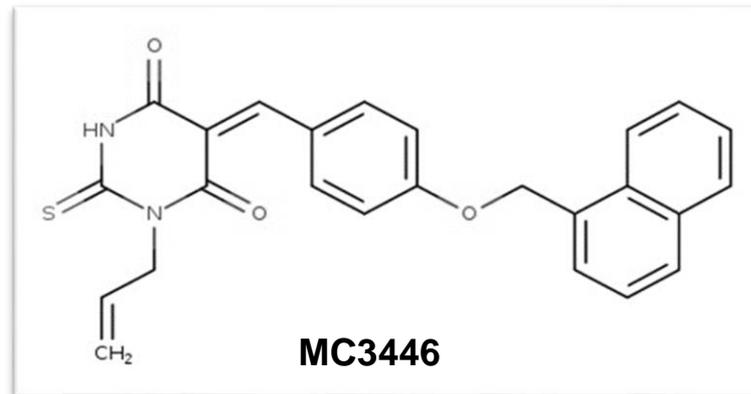


**MC3945**

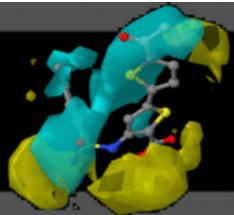


## Tiobarbiturati

**MC3444**

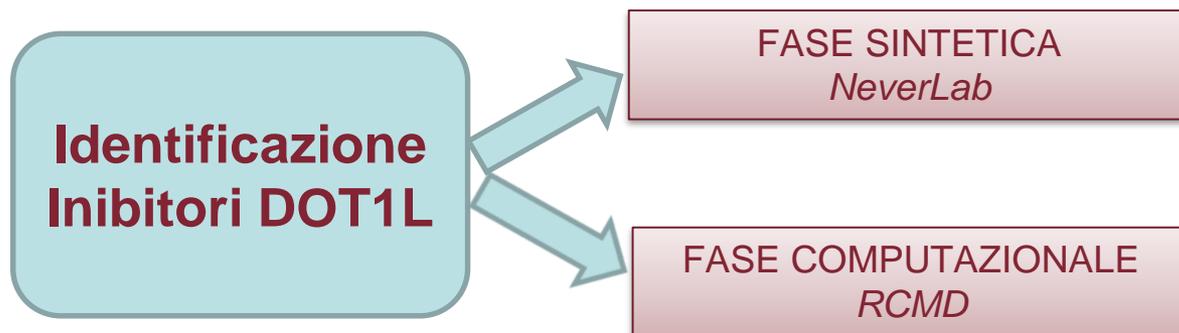


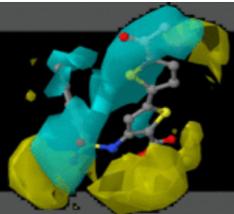
**MC3446**



# Procedura sperimentale

by [www.RCMD.it](http://www.RCMD.it)

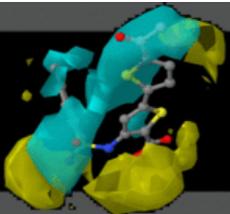




# Procedura sperimentale

by [www.RCMD.it](http://www.RCMD.it)





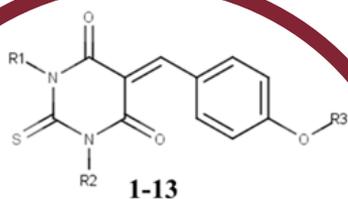
# Sintesi tiobarbiturati sostituiti

by **www.RCMD.it**

Identificazione  
Inibitori DOT1L

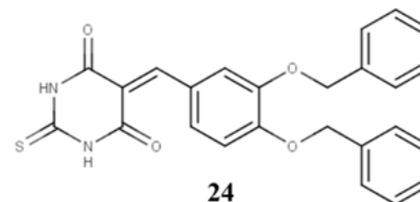
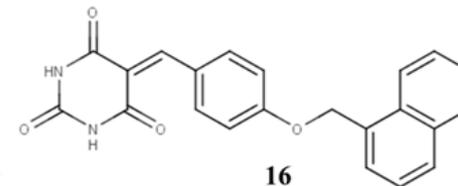
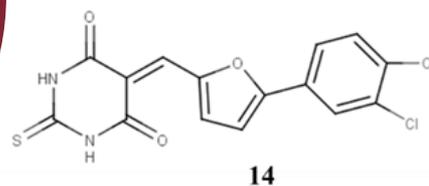
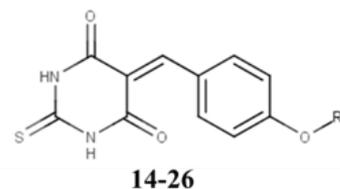
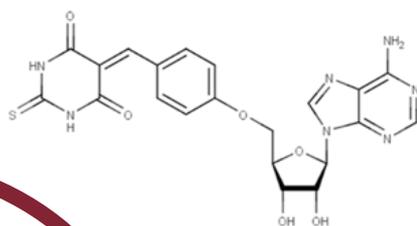
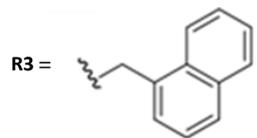
FASE SINTETICA  
*NeverLab*

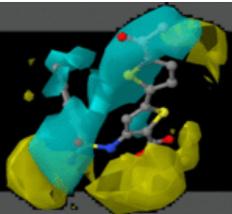
Sintesi  
Tiobarbiturati



R1 = H, Me, Et, Pr, Bu, allil, benzil

R2 = H, Me, Et, Pr, Bu, allil, Ph, benzil



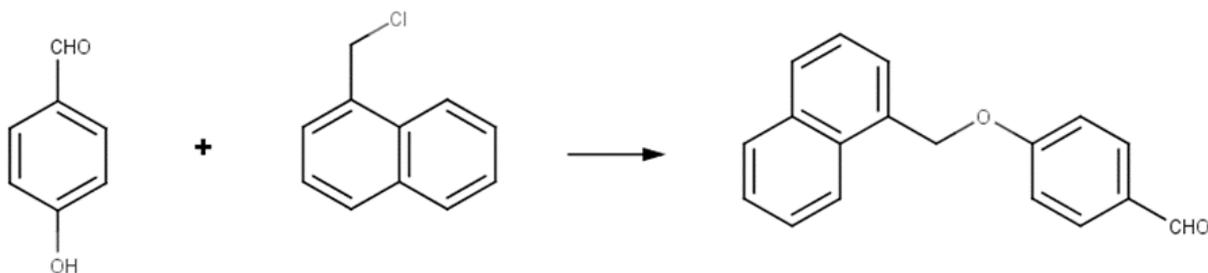


# Sintesi tiobarbiturati sostituiti /1

by [www.RCMD.it](http://www.RCMD.it)

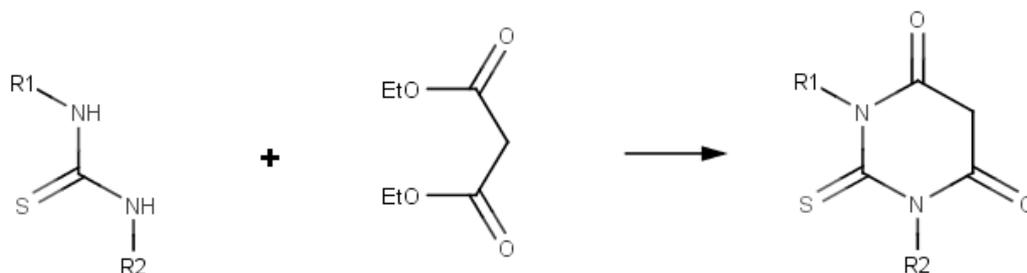
Sintesi di due *sintoni*:

- 4-oxobenzaldeidi diversamente sostituite.



$K_2CO_3$ , NaI,  
95° C,  $CH_3CN$  *dry*, 3h.

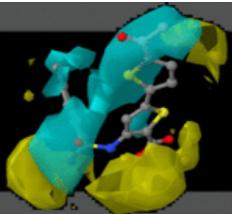
- Acidi 2-tiobarbiturici N-sostituiti e N,N-disostituiti.



Na, EtOH *dry*, 6 h.

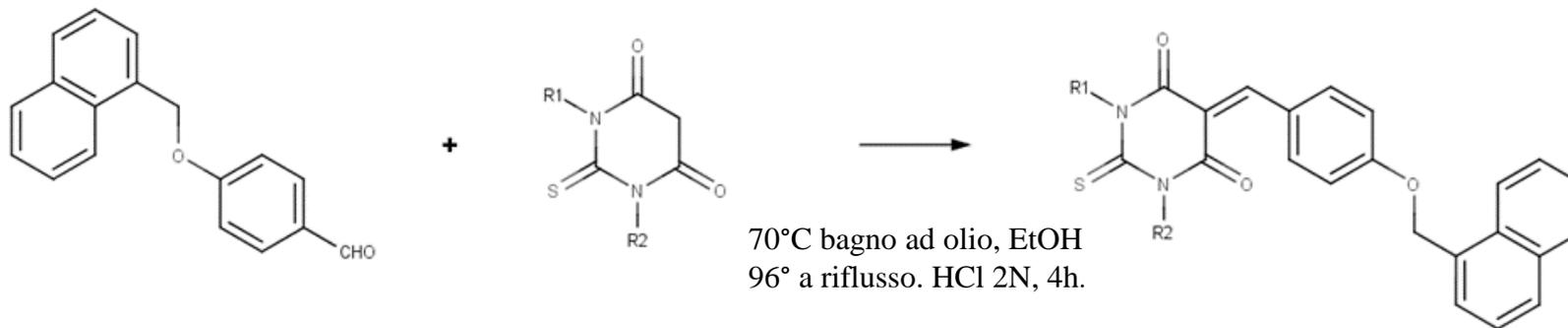
$R_1 = H, Me, Et, n-Pr, All, n-Bu, Bn$

$R_2 = H, Me, Et, n-Pr, All, n-Bu, Bn$

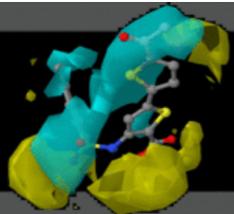


# Sintesi tiobarbiturati sostituiti /2

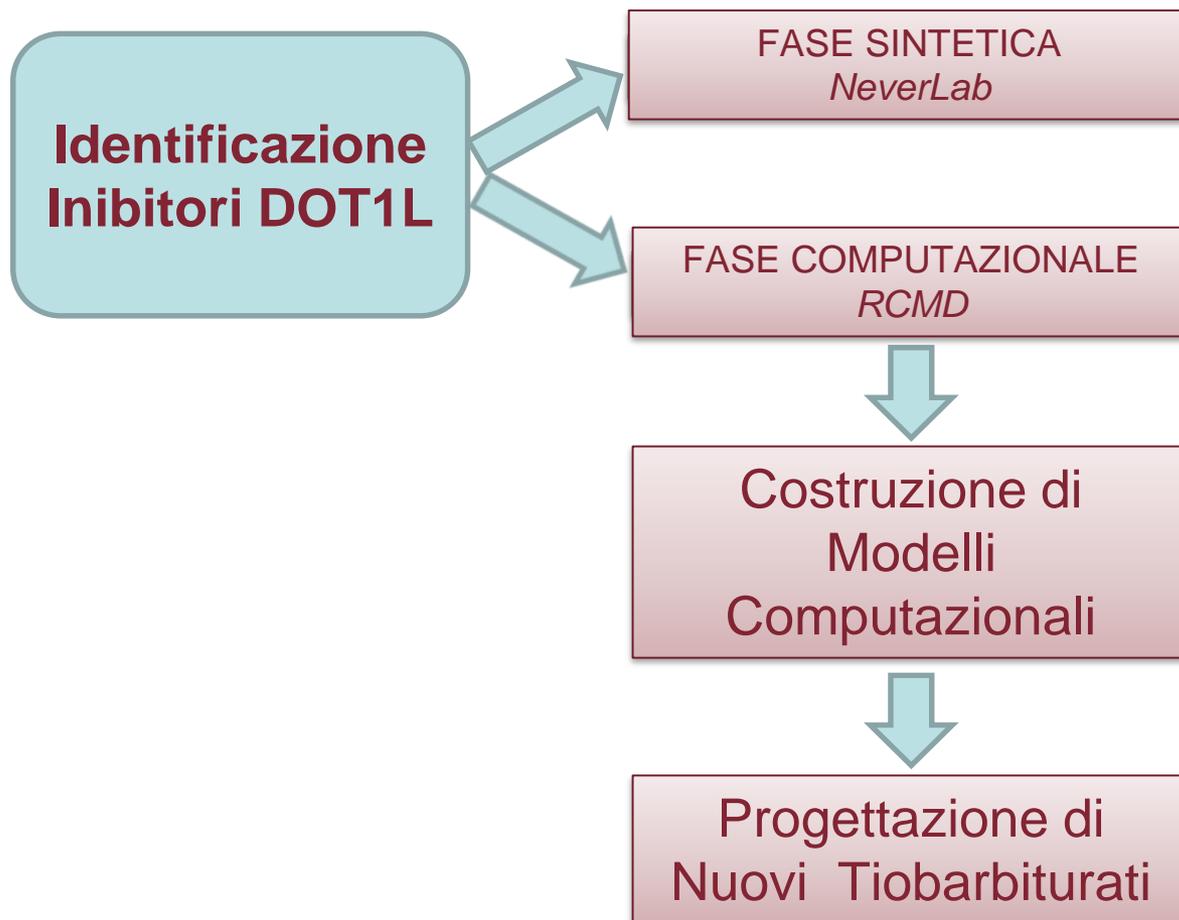
by **www.RCMD.it**

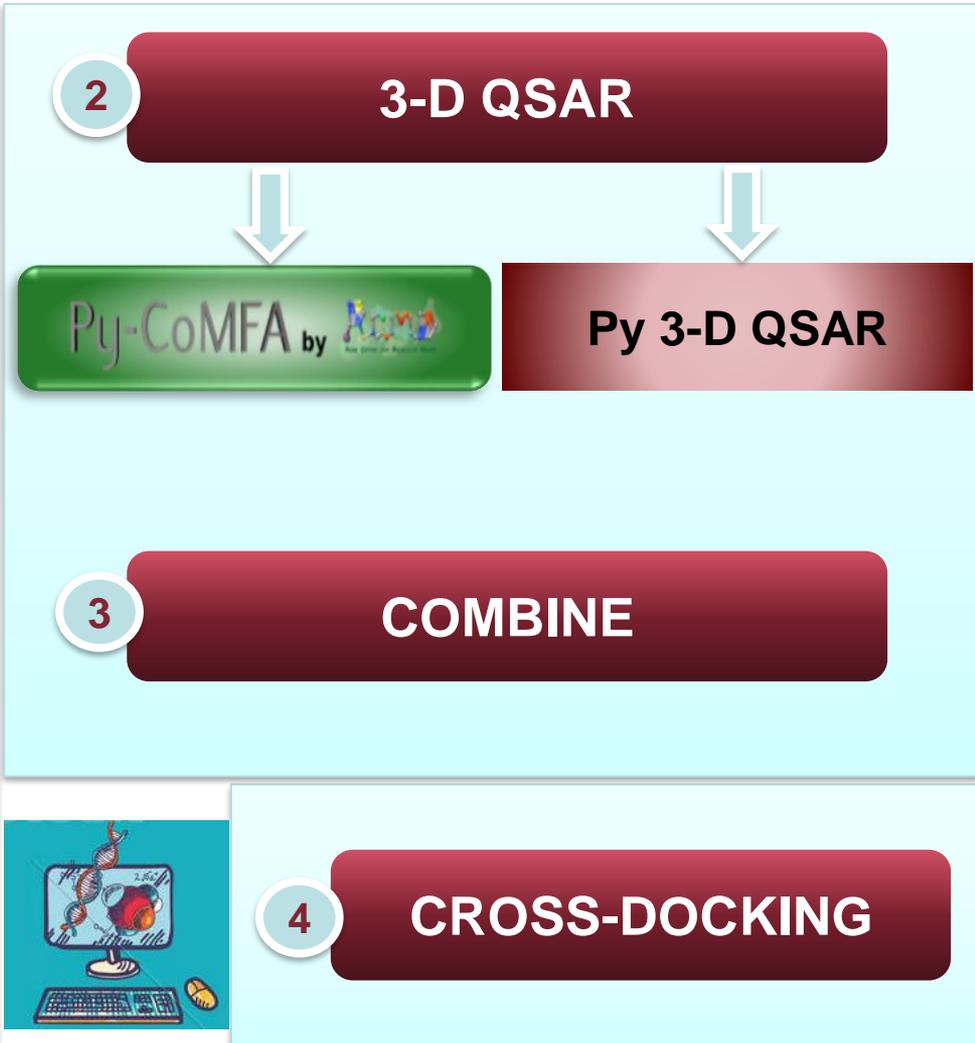
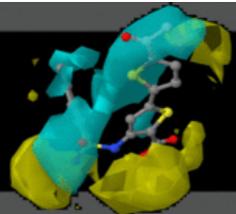


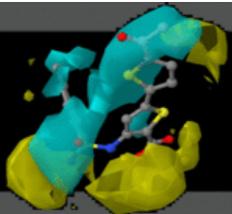
Composto	Sigla	R <sub>1</sub>	R <sub>2</sub>	PM	p.f. °C	Resa (%)
1	MC3425	Me	H	402.47	283-284	83
2	MC3426	Et	H	416.49	>299	82
3	MC3427	n-Pr	H	430.52	>299	88
4	MC3428	Bn	H	478.56	>299	89
5	MC3440	Et	Et	444.55	>299	100
6	MC3442	Bn	Bn	568.68	>299	71
7	MC3445	n-Bu	H	444.55	281-282	77
8	MC3446	All	H	428.50	>299	83
9	MC3447	Me	Me	416.49	>299	70
10	MC3448	n-Pr	n-Pr	472.60	>299	70
11	MC3449	n-Bu	n-Bu	500.65	>299	41
12	MC3454	All	All	468.57	>299	71
13	MC3944	Bn	H	464.53	>299	79



# Procedura sperimentale







# Costruzione di un modello QSAR

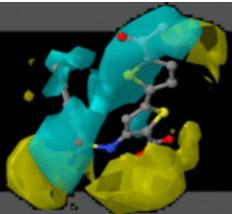
1

## QSAR

Algoritmi di Regressione e Cross-validazione		$r^2$	$q^2$
PLS: Partial Least Squares regression	Leave one out	0.97	0.68
Random Forest Regressor	Shuffle split	0.94	0.51
Gradient Boosting Regressor	Leave one out	0.99	0.67

Algoritmo di Classificazione	<i>Accuracy score</i>	<i>MCC</i>
Logistic Regression	0.92	0.83



# Three Dimensional Quantitative Structure-Activity Relationship

by [www.RCMD.it](http://www.RCMD.it)

2

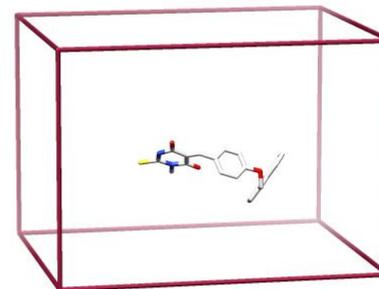
## 3-D QSAR

Training Set

Inibitori DOT1L  
da PDB

External validation  
test set

Tiobarbiturati



Py-CoMFA by 

Py 3-D QSAR

[www.3d-qsar.com](http://www.3d-qsar.com)

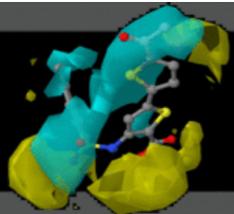
Linea di comando linux

Probe H (6 PCs)

Probe HD (5 PCs)

$r^2$	0,98
$q^2$	0,82
SDEP_pred	1,07

$r^2$	0,96
$q^2$	0,79
SDEP_pred	0,90

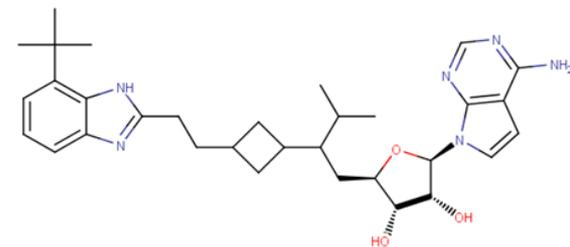
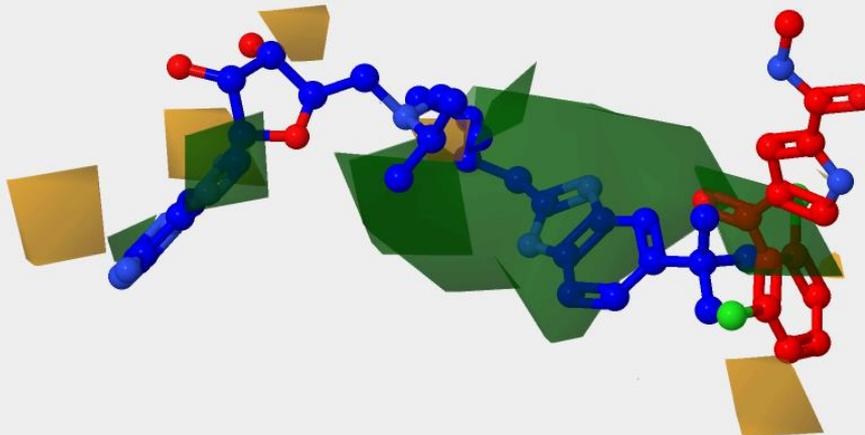


# Mappe 3-D QSAR

Training set

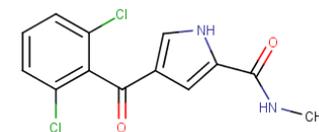
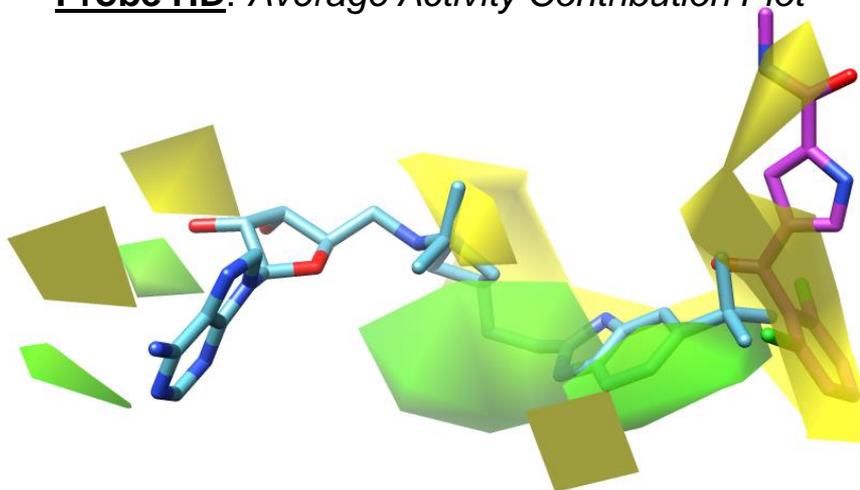
Py-CoMFA by [RCMD](http://www.rcmd.it)

Probe H: Average Activity Contribution Plot



molecola più attiva 4HRA/EPZ-5676  
(10.10  $\mu\text{M}$ )

Probe HD: Average Activity Contribution Plot



molecola meno attiva 5DTM/5F4  
(3.45  $\mu\text{M}$ )

Py 3-D QSAR



# COMparative BINding Energy

3

## COMBINE

Training Set

Complessi  
DOT1L da PDB

External validation  
test set

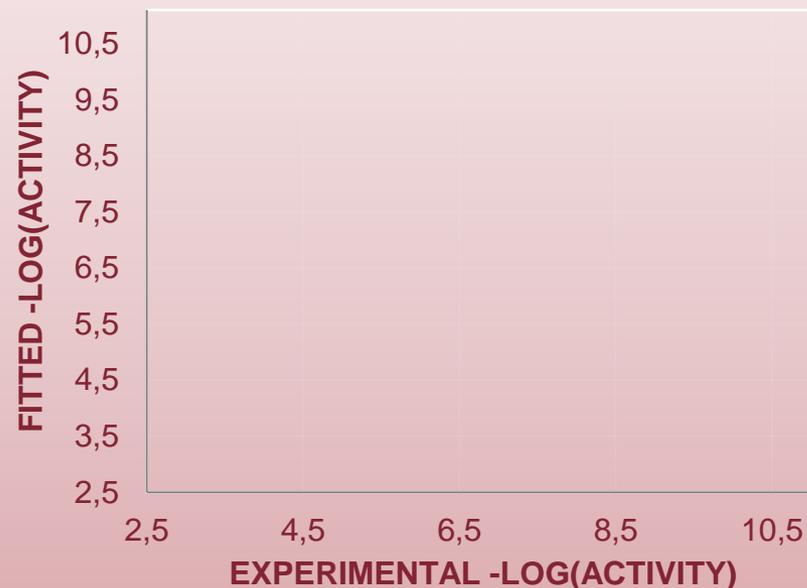
Lock/Key  
Tiobarbiturati

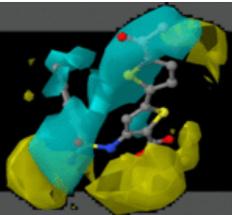
Interazioni analizzate:  
STE-ELE-DRY-HB

### DRY-HB (4 PCs)

$r^2$	0,96
$q^2$	0,79
SDEP_pred	0,90

### Fitting PC4



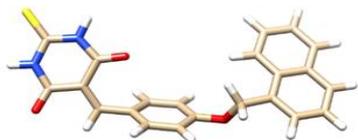


# Molecular Docking

4

## CROSS-DOCKING

26 Tiobarbiturati  
(Key)



15  
Lock

1NW3

3QOW

3QOX

3SR4

3SX0

3UWP

4EK9

4EKG

4EKI

4ER5

4EQZ

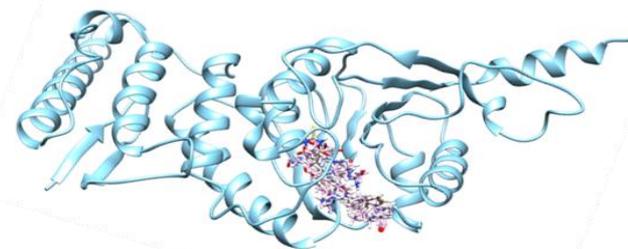
4ER0

4ER6

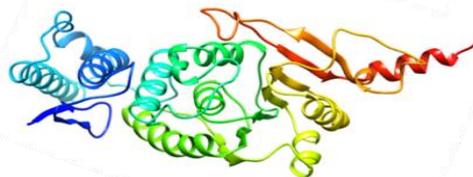
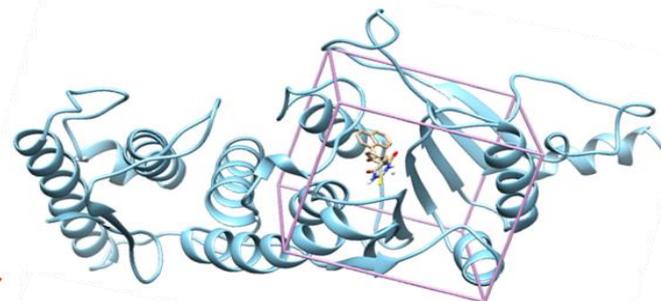
4ER7

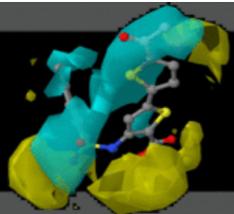
4HRA

Surflex



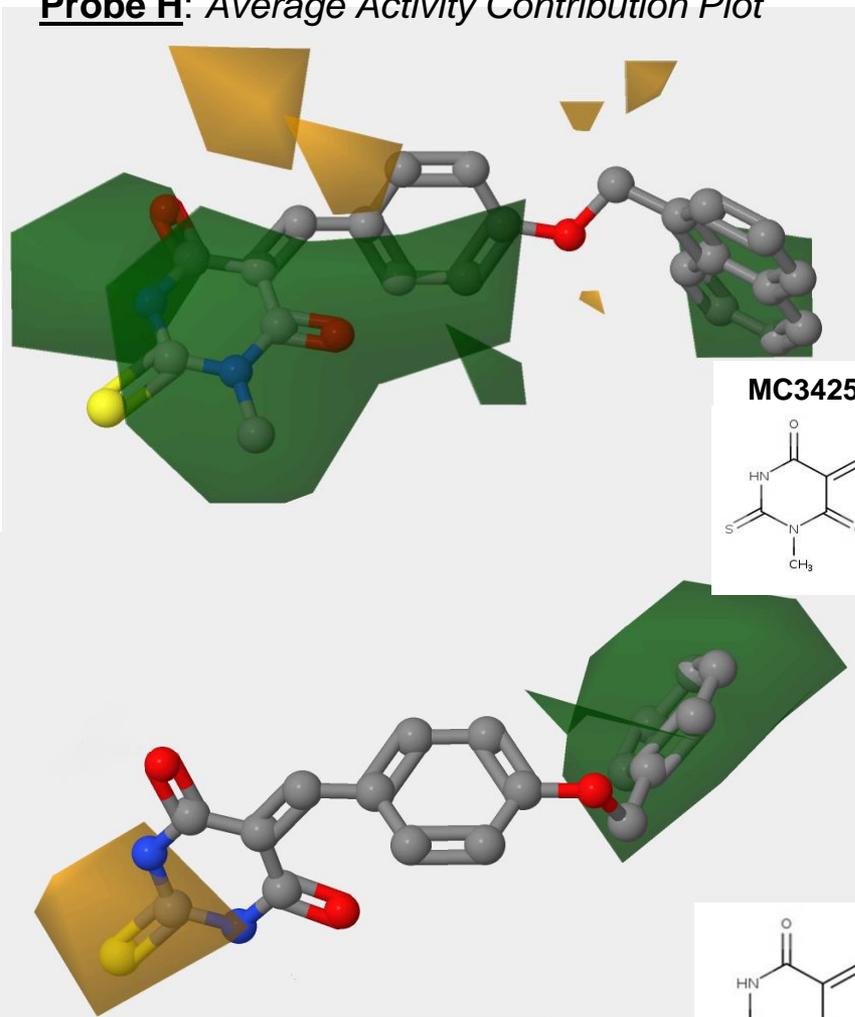
Vina



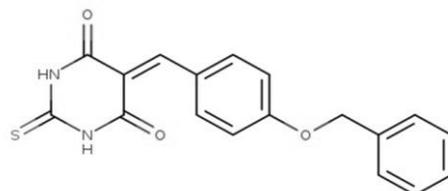
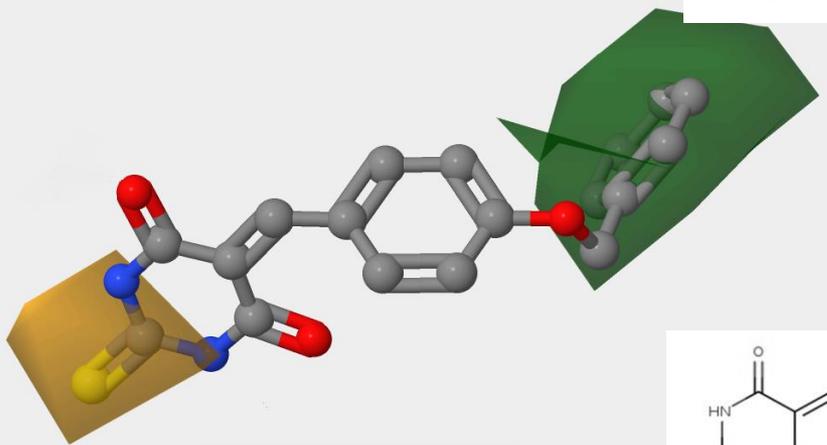
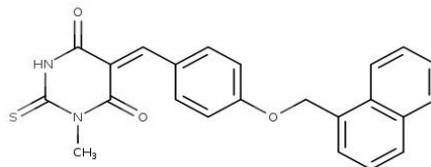


# Mappe 3-D QSAR

**Probe H:** Average Activity Contribution Plot

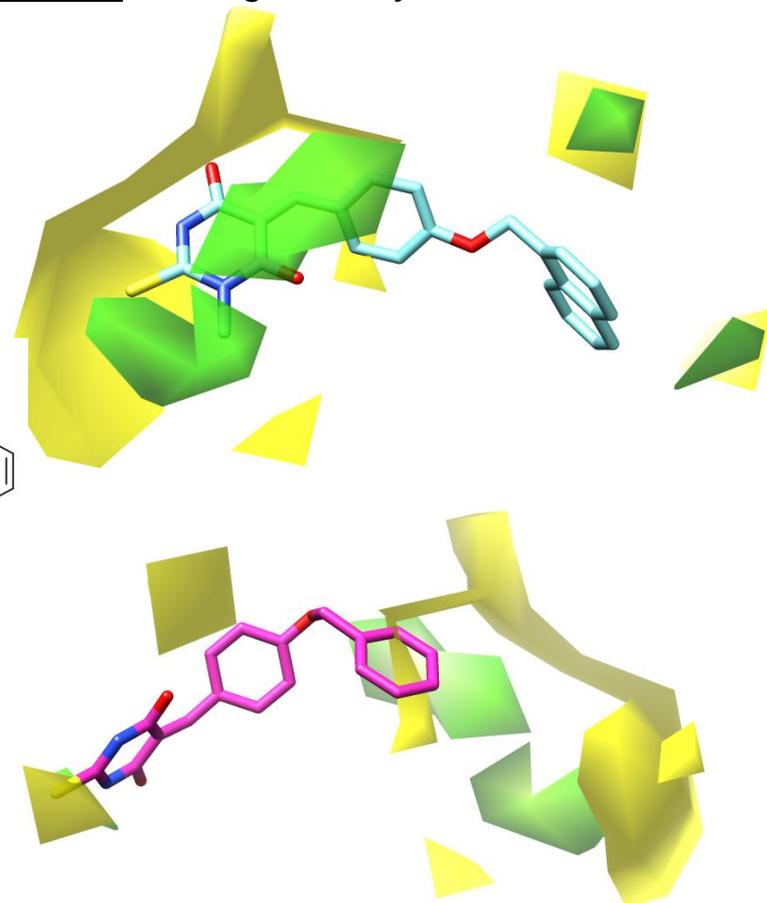


**MC3425 Più attiva (6.87  $\mu\text{M}$ )**



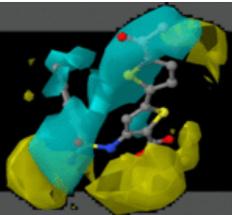
**MC3940 Meno attiva (3.77  $\mu\text{M}$ )**

**Probe HD:** Average Activity Contribution Plot

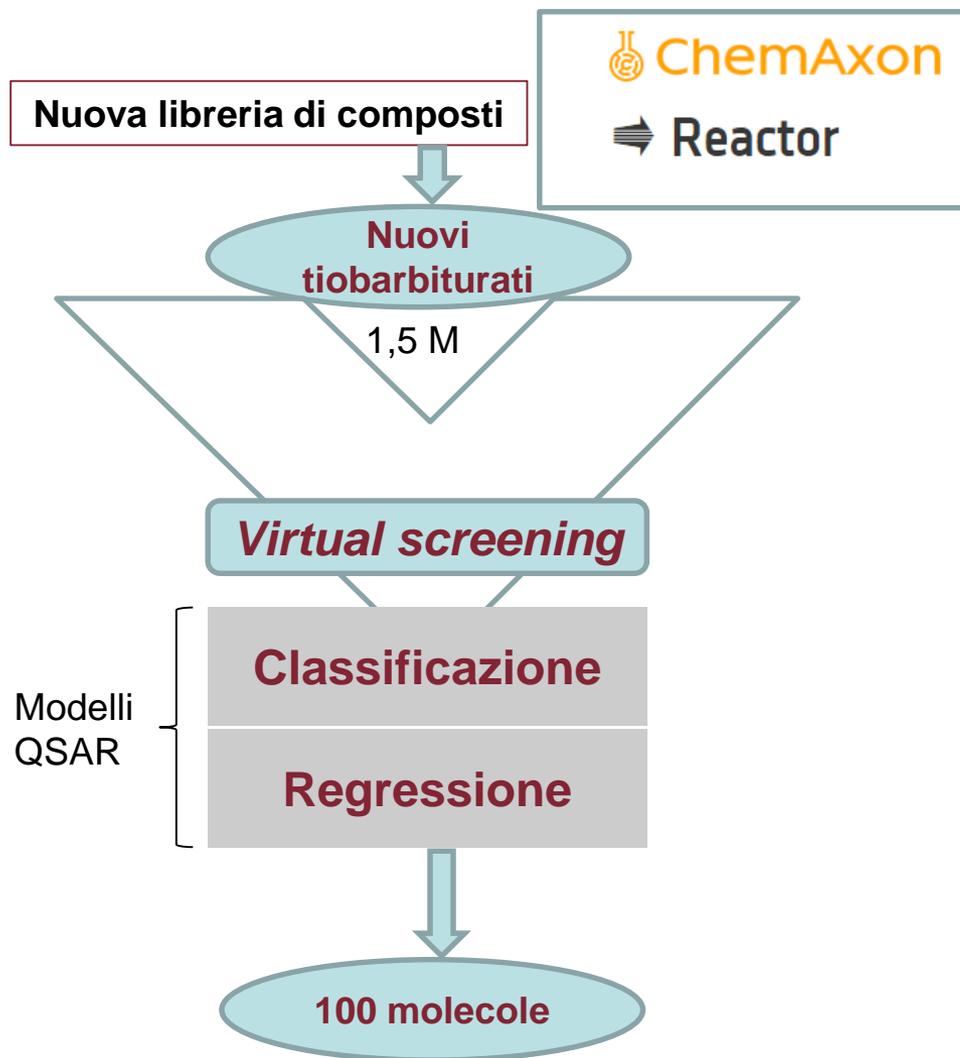


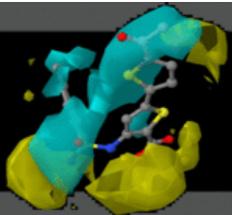
Py-CoMFA by 

Py 3-D QSAR



# Virtual screening

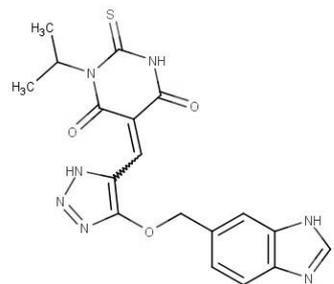
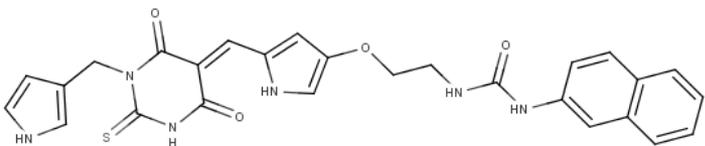




# Predizione attività

by [www.RCMD.it](http://www.RCMD.it)

100 molecole

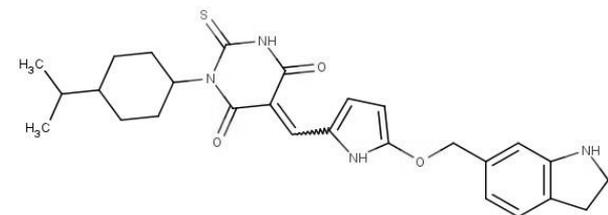
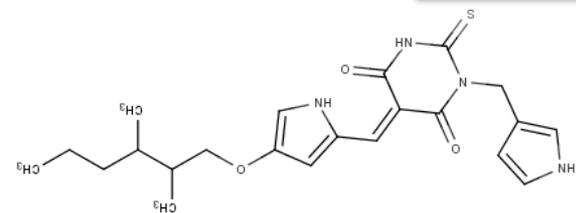


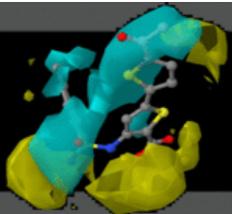
Prediction set

DOCKING

3-D QSAR

COMBINE

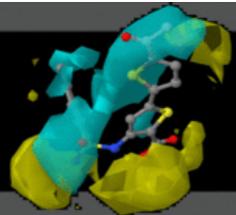




# Conclusioni

by [www.RCMD.it](http://www.RCMD.it)

- I modelli generati risultano validi per la robusta attività predittiva nell'ambito della progettazione di nuovi inibitori DOT1L con lo *scaffold* dei **tiobarbiturati**.
- Inoltre, le 100 migliori molecole predette, grazie ai modelli di classificazione e regressione, e all'applicazione delle metodologie di 3-D QSAR e COMBINE, risultano promettenti per lo studio di una strategia sintetica applicabile in laboratorio.



*Grazie per l'attenzione*