PROGETTAZIONE E SINTESI DI NUOVI INIBITORI DELL'ISTONE METILTRANSFERASI DOT1L

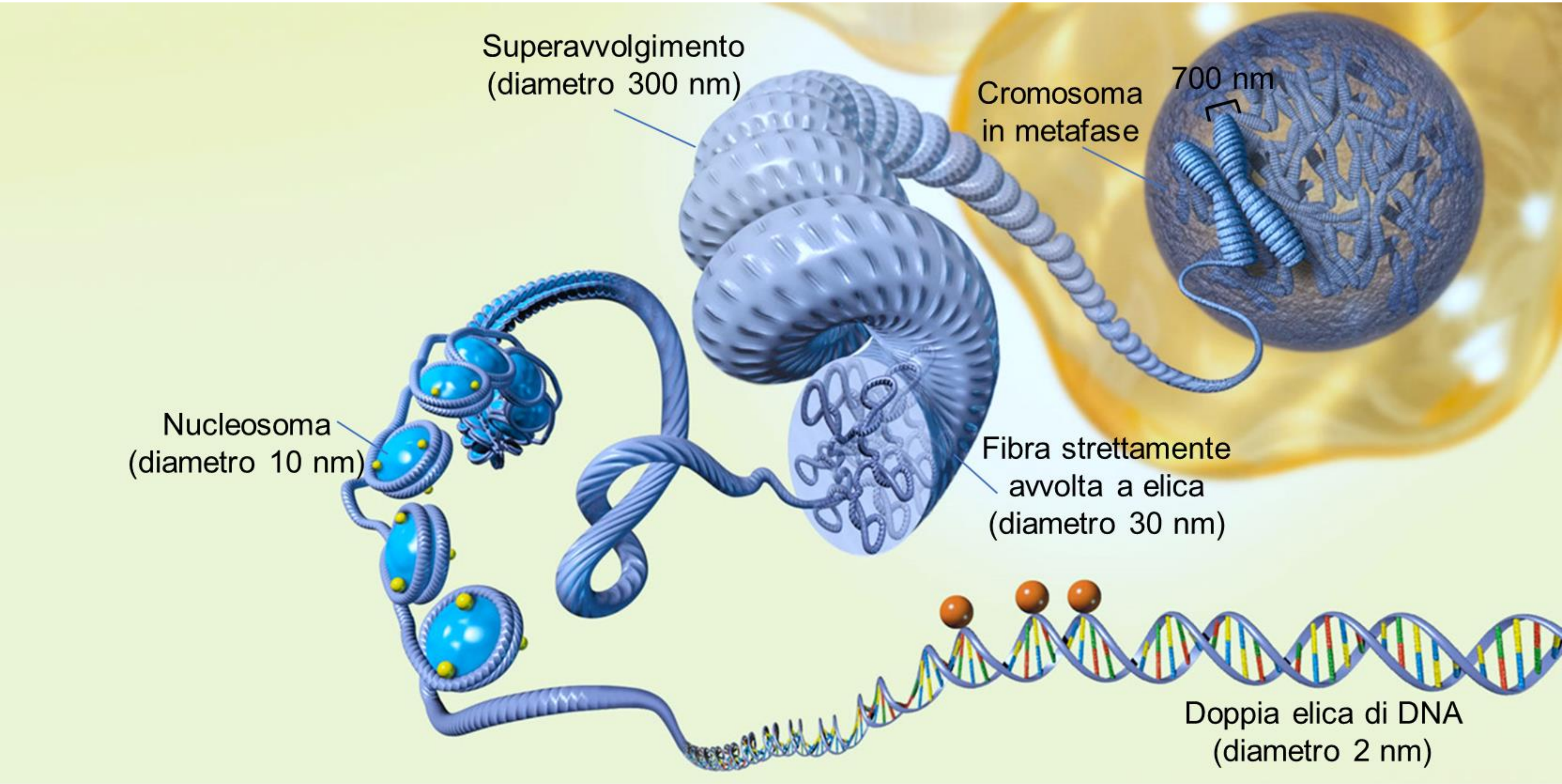
SAPIENZA
UNIVERSITÀ DI ROMA

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

Laureanda: Anna Sarubbi
Matricola: 1243388

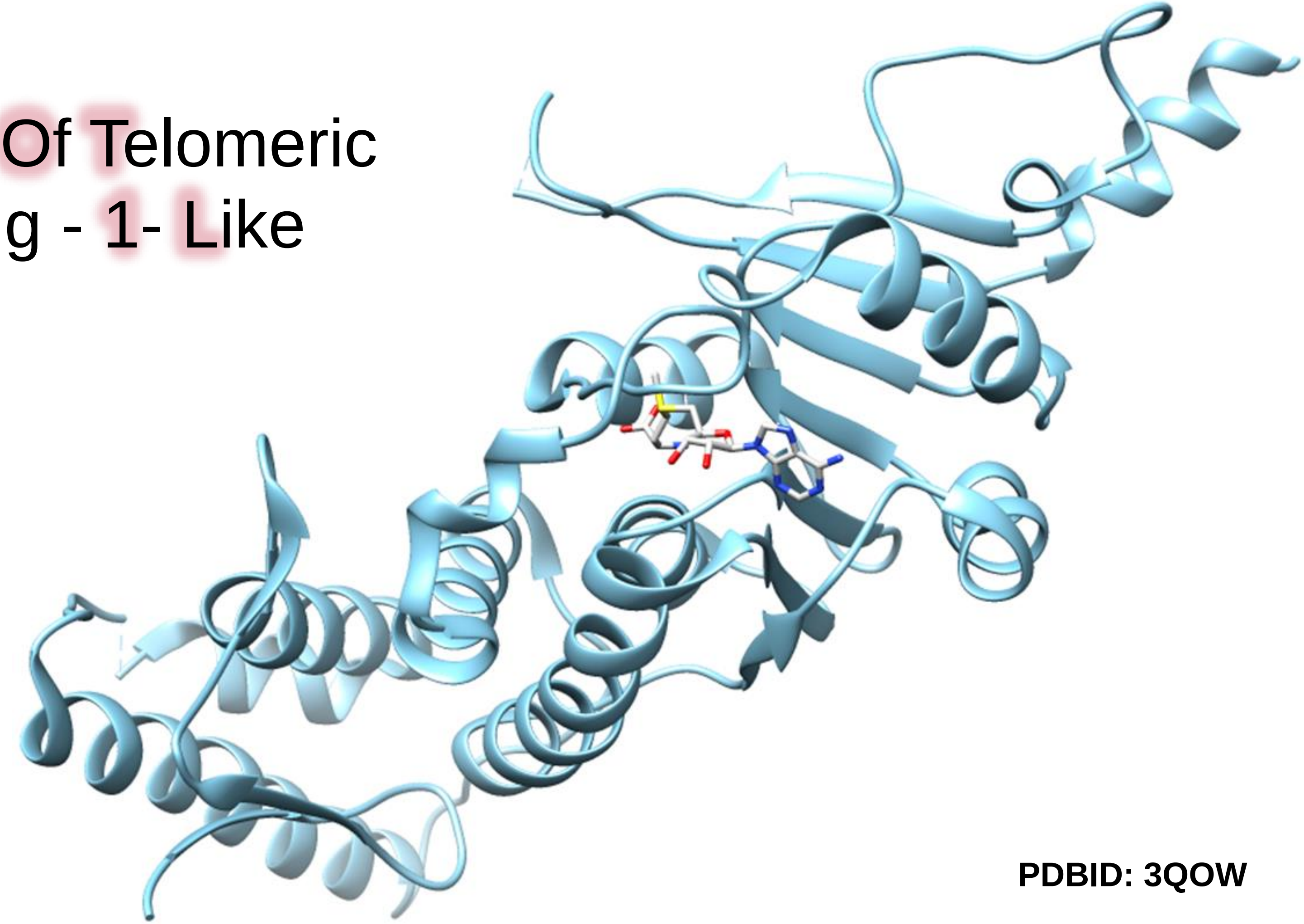
Relatore: Prof. Antonello Mai
Correlatore: Prof. Rino Ragno

EPIGENETICA


Superavvolgimento
(diametro 300 nm)
Cromosoma
in metafase
700 nm
Nucleosoma
(diametro 10 nm)
Fibra strettamente
avvolta a elica
(diametro 30 nm)
Doppia elica di DNA
(diametro 2 nm)

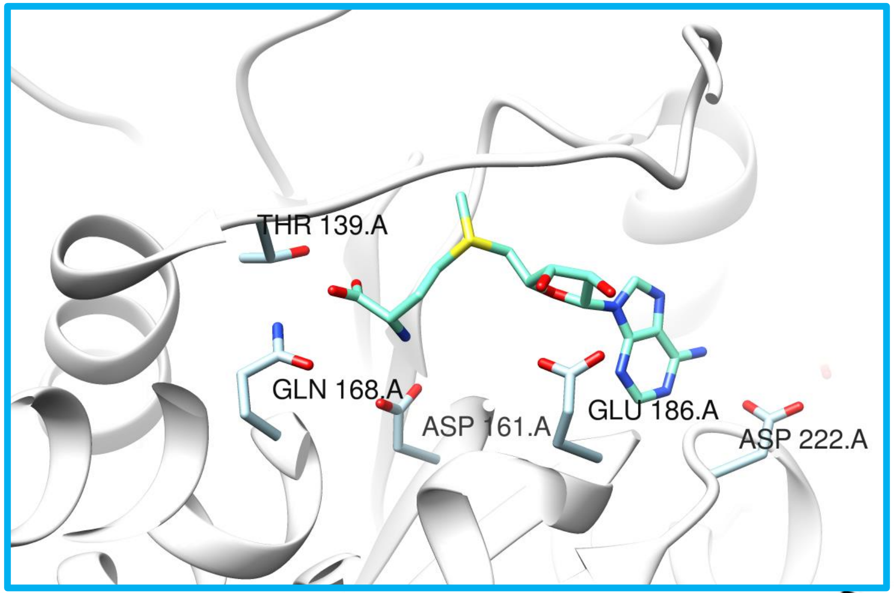
DOT1L

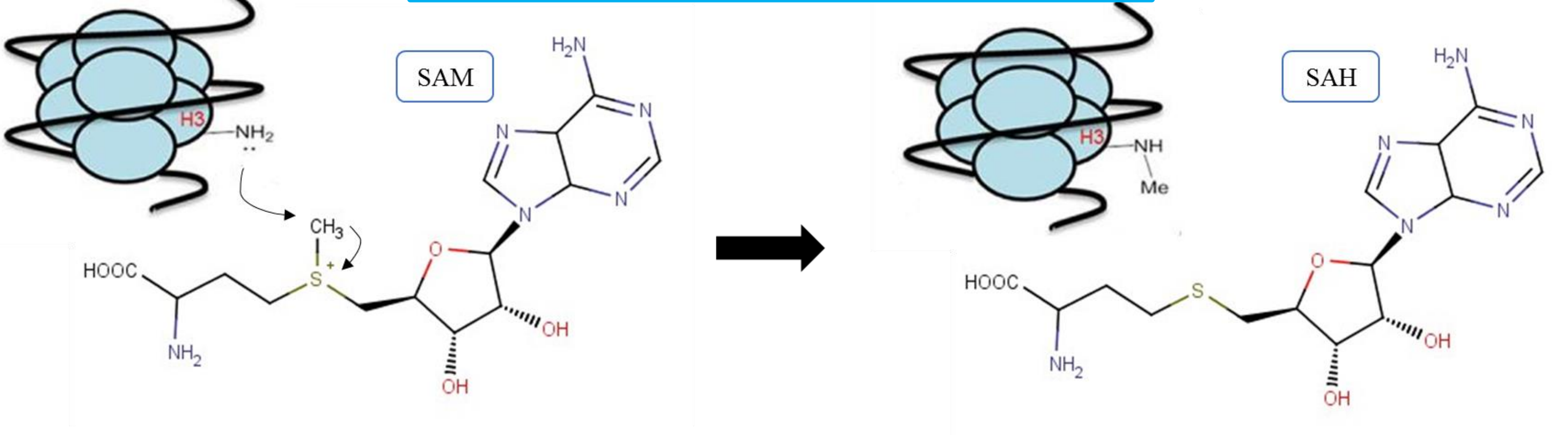
Disruptor Of Telomeric silencing - 1- Like



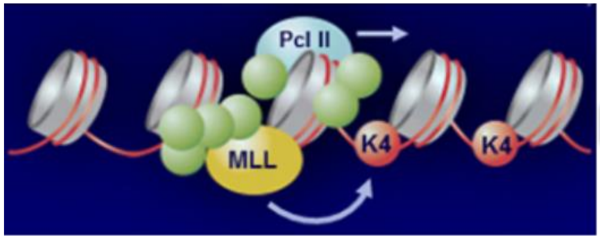
PDBID: 3QOW

MECCANISMO CATALITICO


THR 139.A
GLN 168.A
ASP 161.A
GLU 186.A
ASP 222.A


SAM
H3
NH2
H2N
N
N
N
N
CH3
S+
HOOC
NH2
O
OH
OH
SAH
H3
NH
Me
H2N
N
N
N
N
S
HOOC
NH2
O
OH
OH

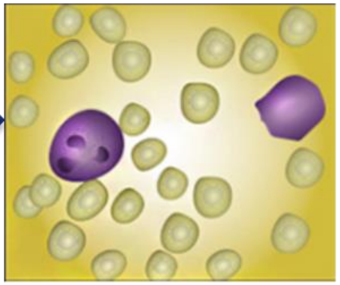
NO Fusione MLL


Pol II
MLL
K4
K4

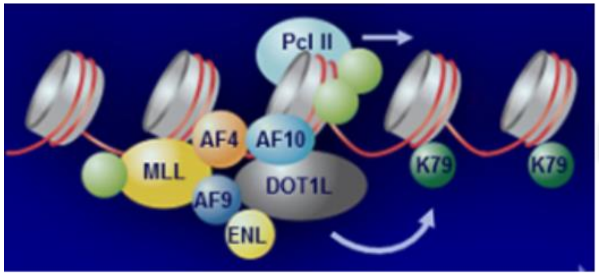
Normale metilazione di H3K79

Normale espressione genica

Differenziamento



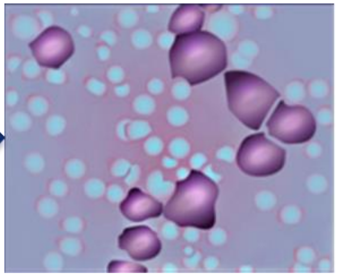
Fusione MLL


Pol II
AF4
AF10
MLL
AF9
DOT1L
ENL
K79
K79

Aberrante metilazione di H3K79

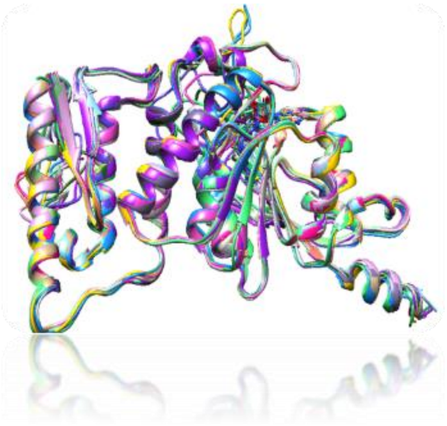
Aberrante attivazione genica (es. HOXA9, MEIS1)

Leucemogenesi

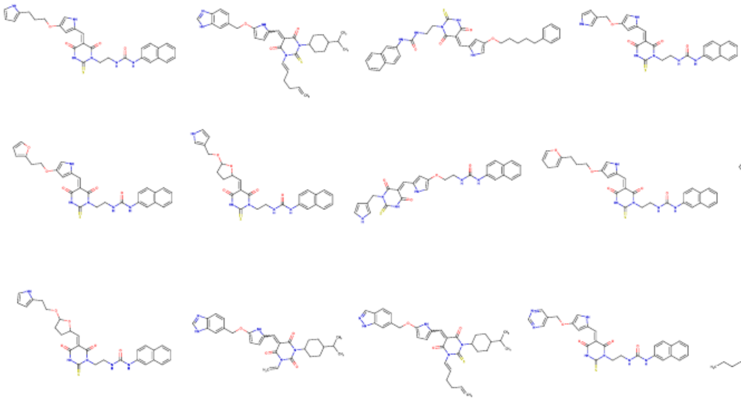


SCOPO DELLA TESI

Raccolta dati DOT1L

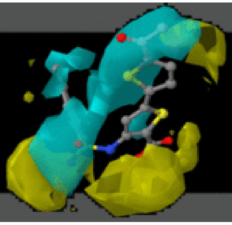


Generazione e applicazione modelli



Progettazione nuove molecole

Sintesi

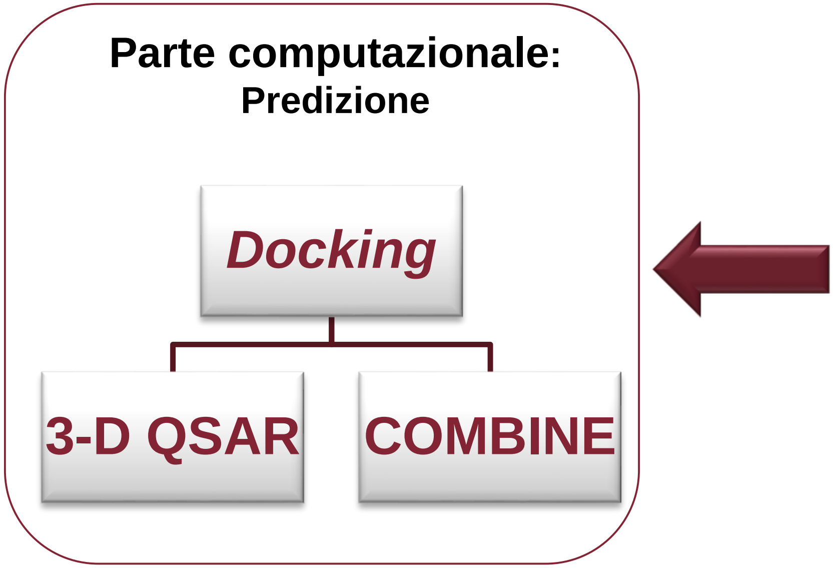


PROCEDURA SPERIMENTALE

by www.RCMD.it

Parte sintetica

Sintesi molecole a struttura tiobarbiturica

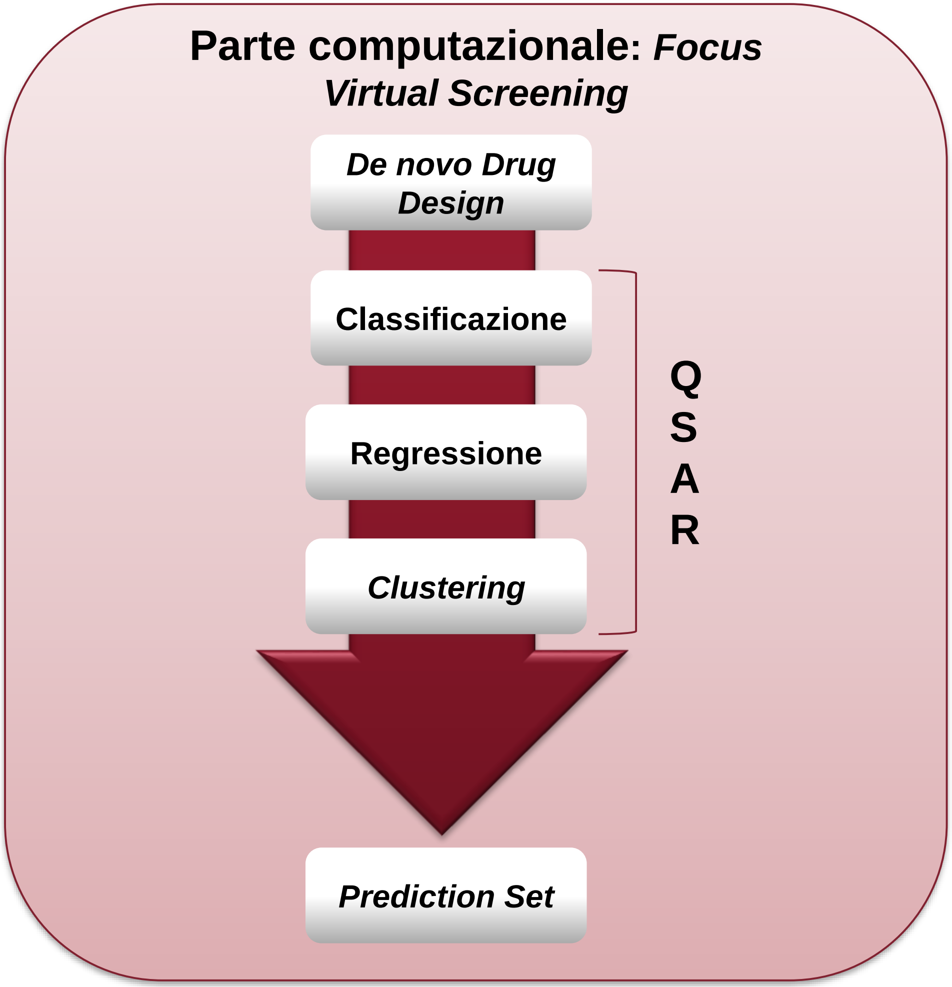


Parte computazionale: Predizione

Docking

3-D QSAR

COMBINE



Parte computazionale: *Focus Virtual Screening*

De novo Drug Design

Classificazione

Regressione

Clustering

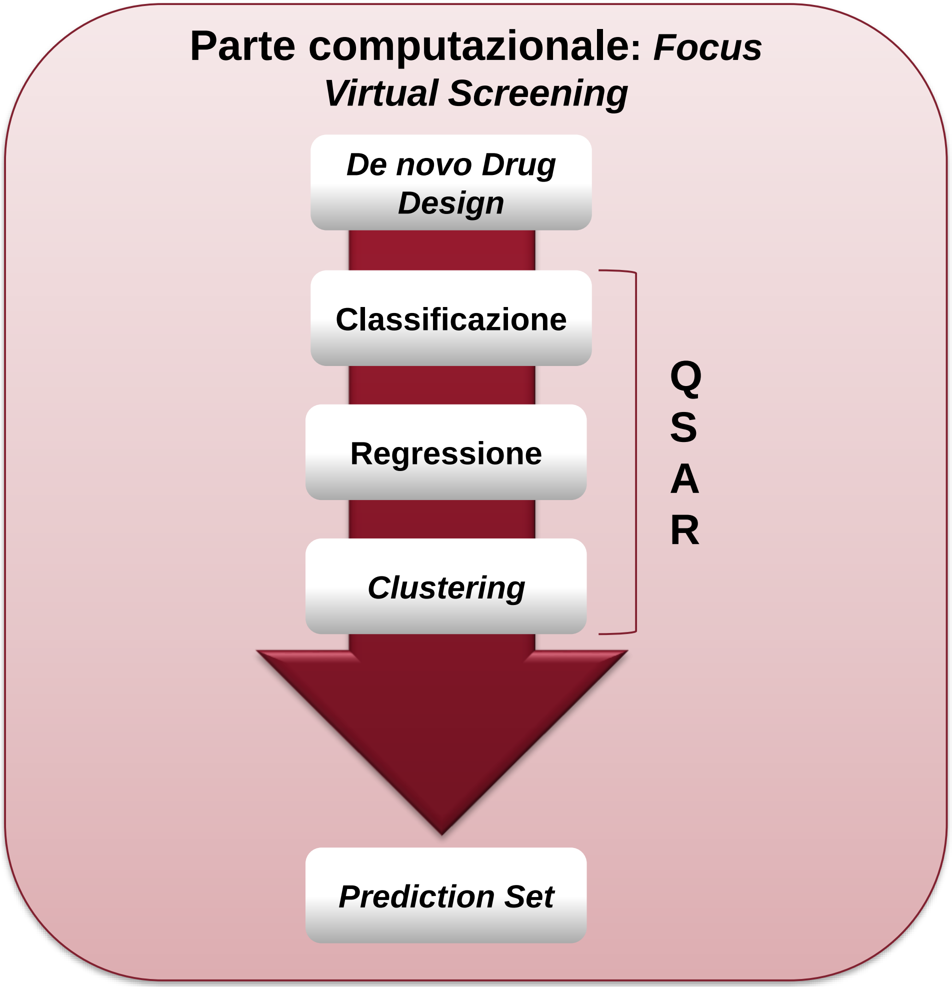
QSAR

Prediction Set

PROCEDURA SPERIMENTALE/1

Parte sintetica

Sintesi molecole a struttura tiobarbiturica



Parte computazionale: *Focus Virtual Screening*

De novo Drug Design

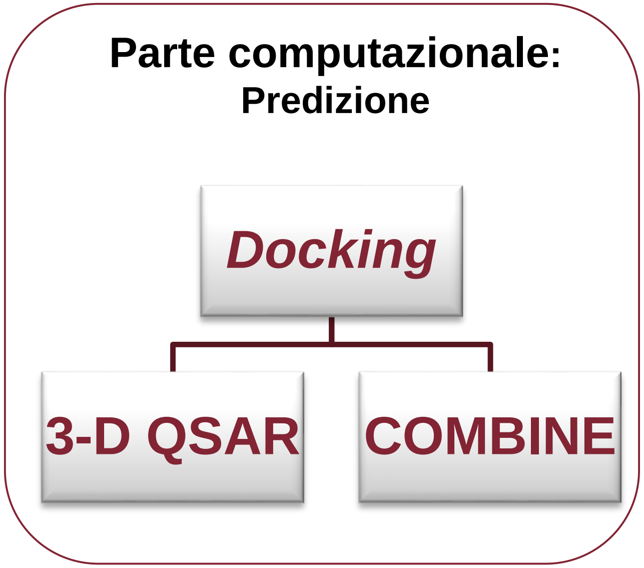
Classificazione

Regressione

Clustering

QSAR

Prediction Set



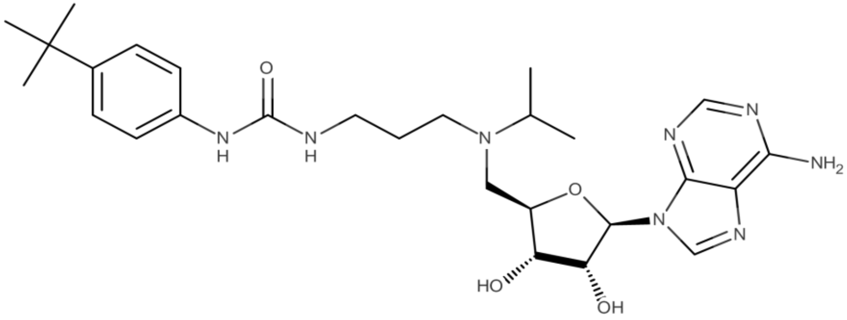
Parte computazionale: Predizione

Docking

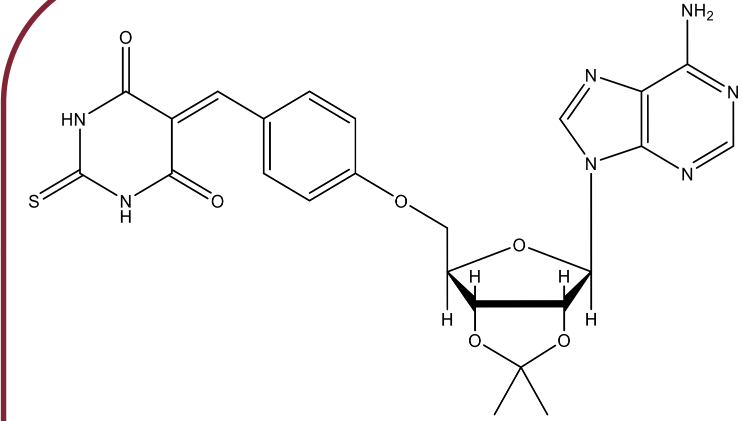
3-D QSAR

COMBINE

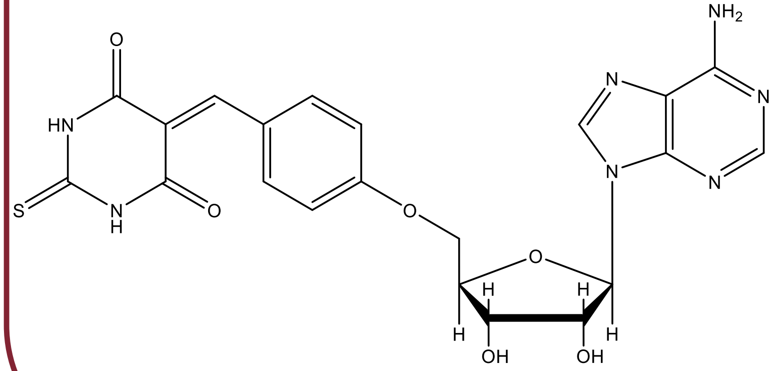
INIBITORI DOT1L


O
N
H
N
H
N
O
N
N
NH2
N
N
HO
OH

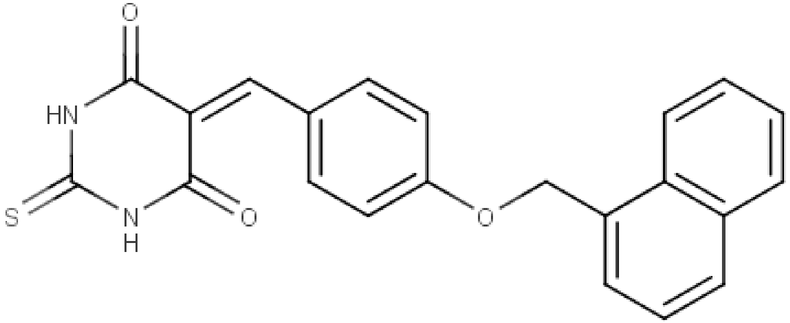
SYC-522 Ki = 0,5 nM e EC_{50} = 4,4 µM


O
HN
S
N
H
O
O
O
H
H
H
H
O
O
NH2
N
N
N
N

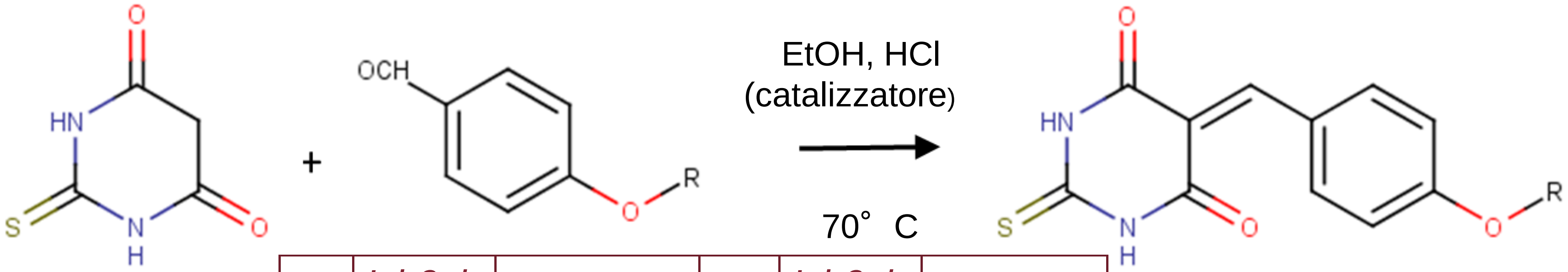
MC3938 % inib (50 µM) = 59,32

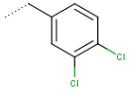
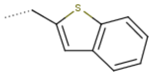
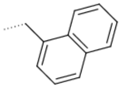
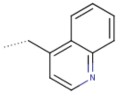
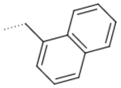
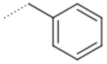
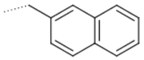
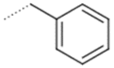
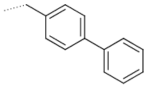
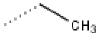
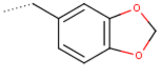
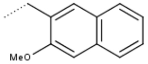
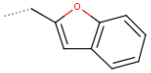

O
HN
S
N
H
O
O
O
H
H
H
H
OH
OH
NH2
N
N
N
N

MC3945 % inib (50 µM) = 94,68

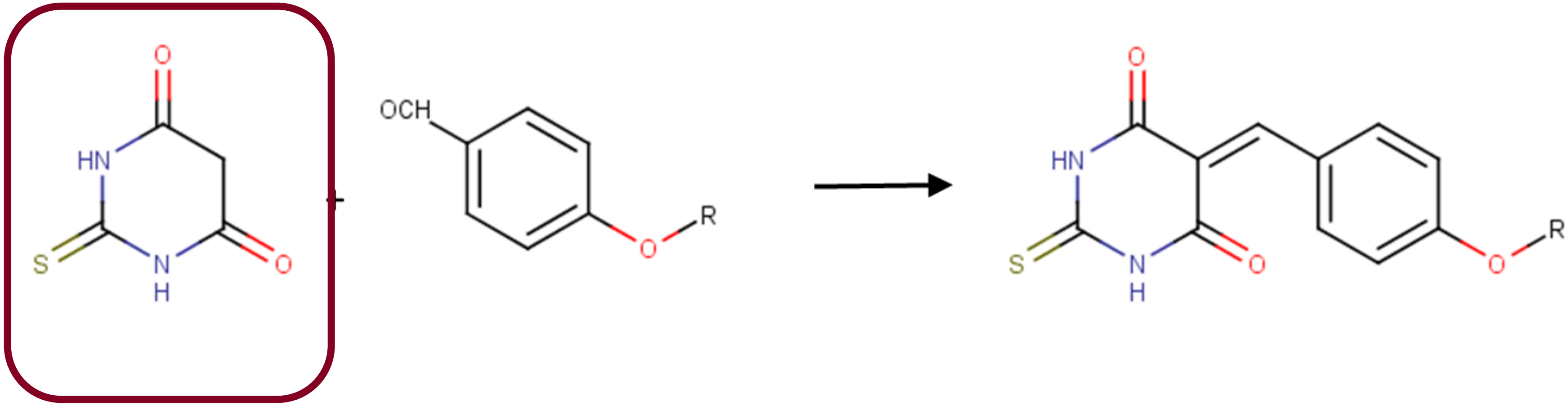


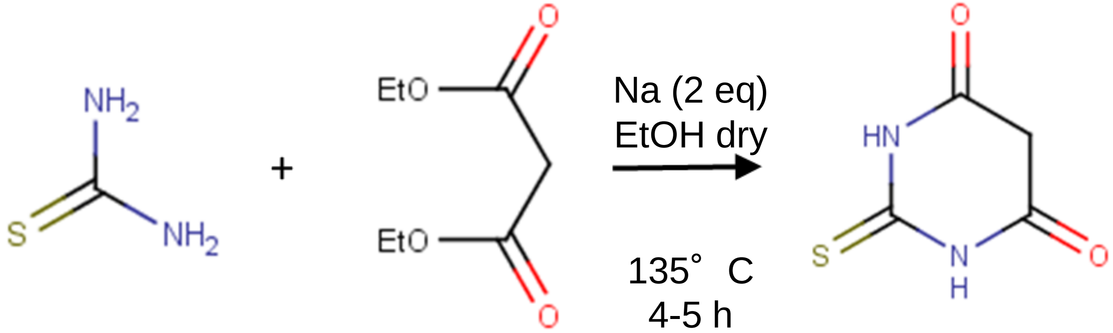
Sintesi Tiobarbiturati non sostituiti


O
HN
S
N
H
O
+
OCH
O
R
EtOH, HCl
(catalizzatore)
70° C
O
HN
S
N
H
O
O
R

	Lab Code	***R***		***Lab Code***	***R***
1	MC3443		**8**	MC3926	
2	MC3444		**9**	MC3927	
3	MC3920		**10**	MC3940	
4	MC3921		**11**	MC3941	
5	MC3922		**12**	MC3942	
6	MC3923		**13**	MC3943	
7	MC3925				

Sintesi acido tiobarbiturico


O
HN
S
N
H
O
+
OCH
O
R
O
HN
S
N
H
O
O
R


NH$_2$
S
NH$_2$
+
EtO
O
EtO
O
O
HN
S
N
H
O

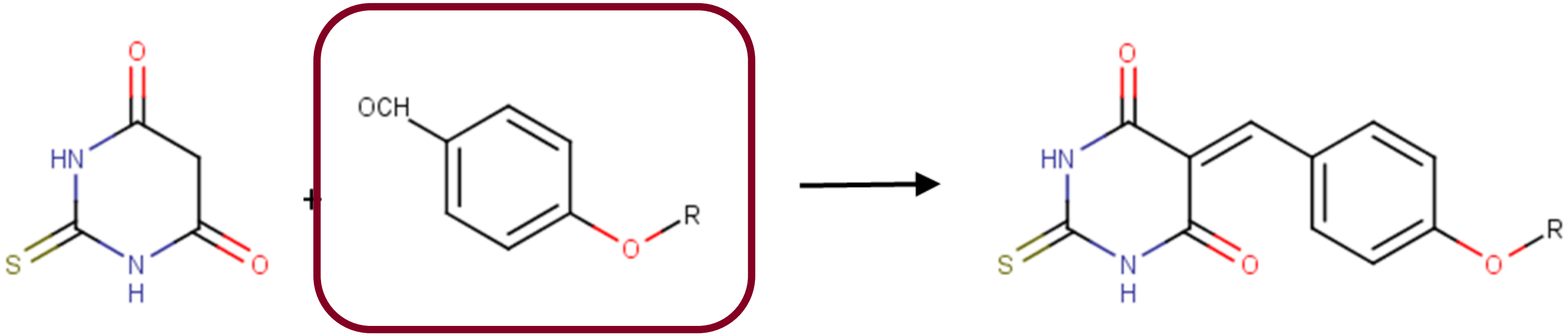
Na (2 eq)
EtOH dry

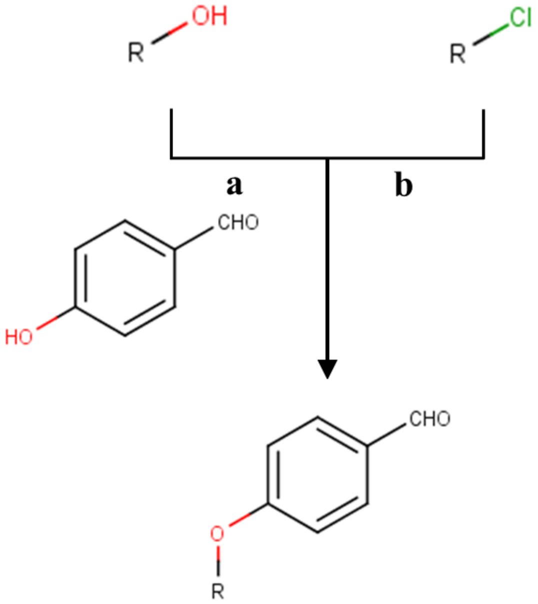
135° C
4-5 h

1 eq

1,1 eq

Sintesi 4-ossibenzaldeidi


O
HN
S
N
H
O
+
OCH
O
R
O
HN
S
N
H
O
O
R


R
OH
R
Cl
a
b
CHO
HO
CHO
O
R

a) alcol, PPh$_3$, DIAD, THF dry, N$_2$, 0° C → rt, O.N. (over night)

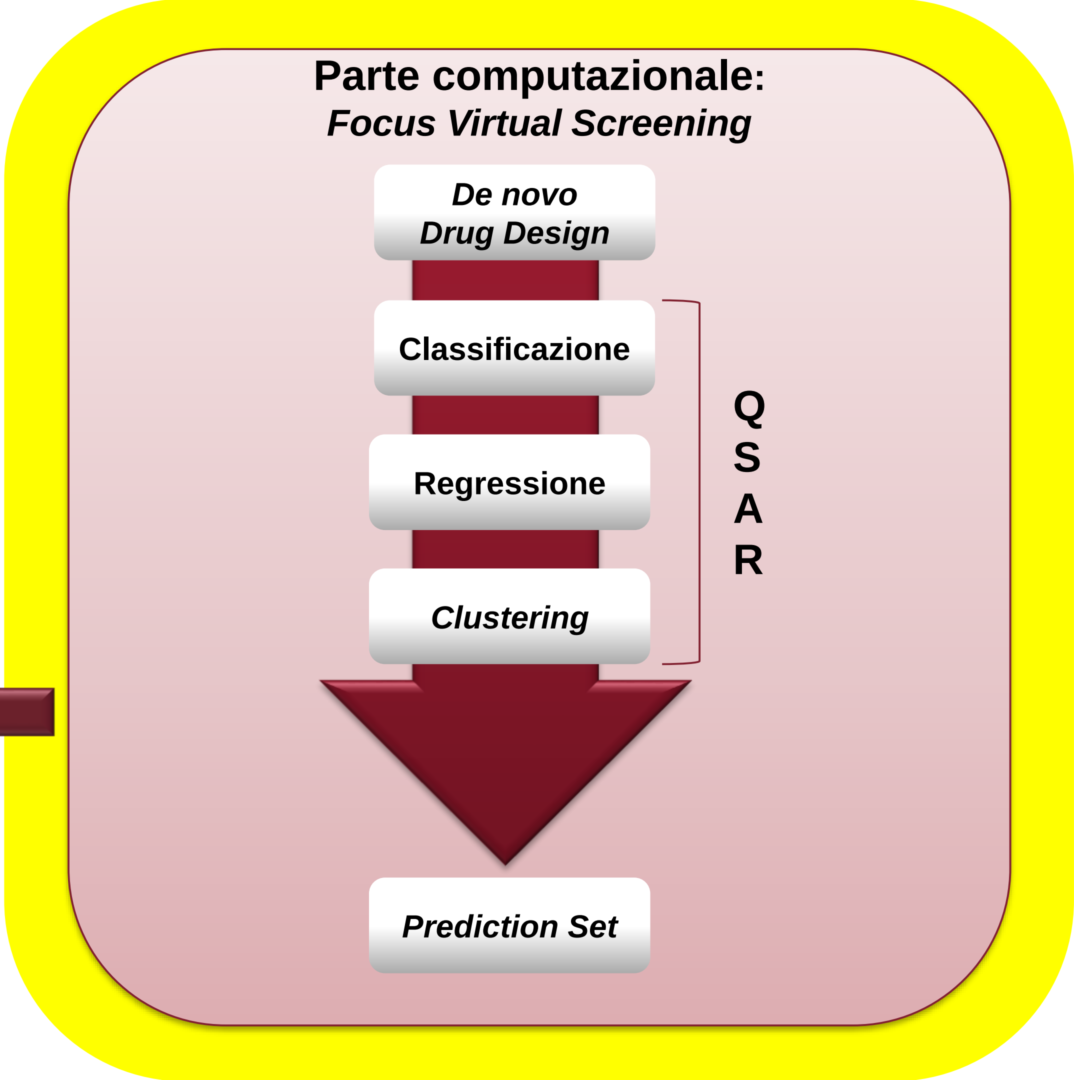
b) cloruro, K_2CO_3, NaI, CH_3CN dry, reflusso, 4h

PROCEDURA SPERIMENTALE/2

by www.RCMD.it

Parte sintetica

Sintesi molecole a struttura tiobarbiturica



Parte computazionale: *Focus Virtual Screening*

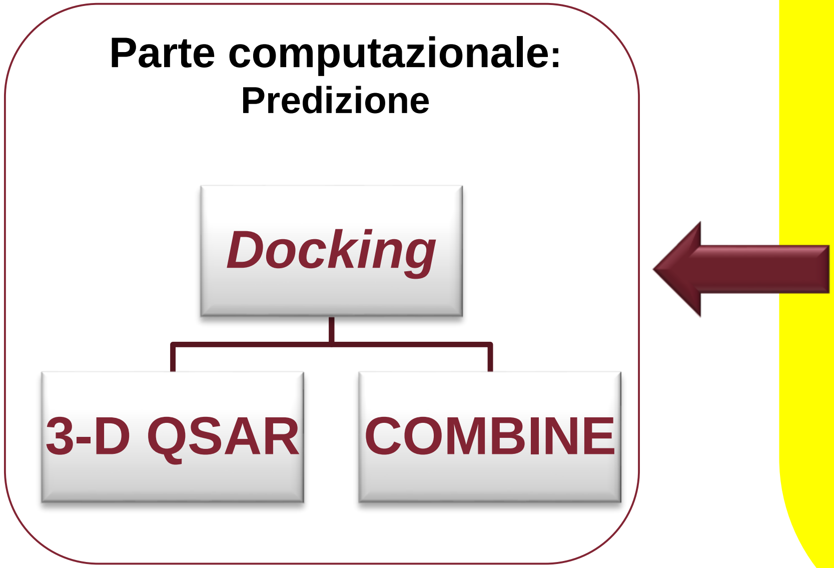
De novo Drug Design

Classificazione

Regressione

Clustering

Prediction Set

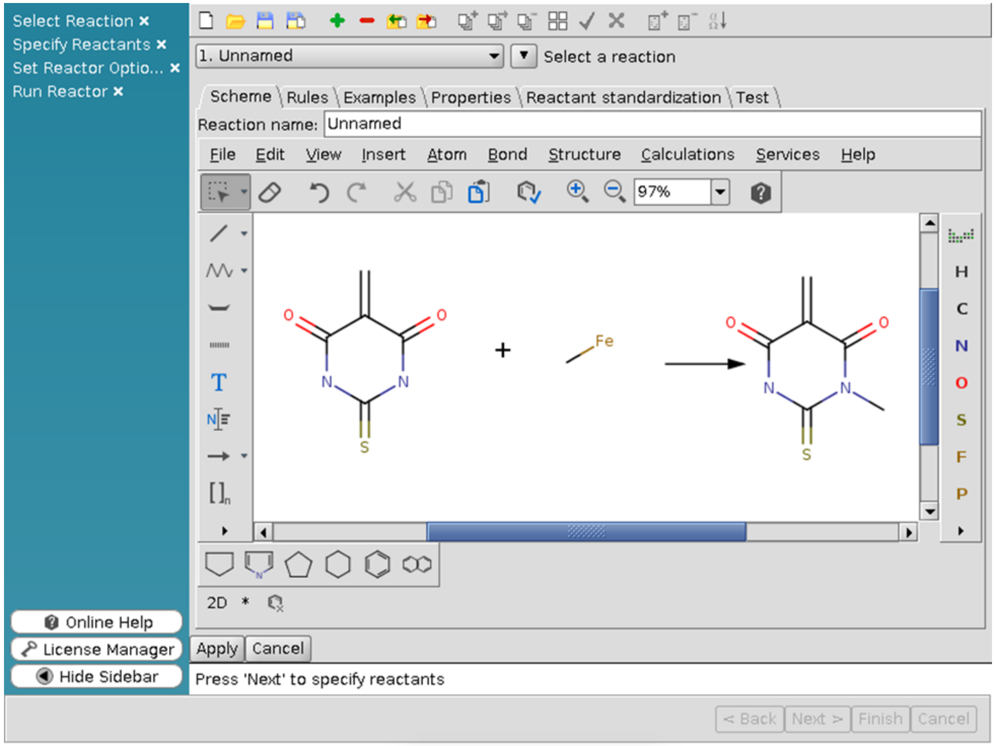


Parte computazionale: Predizione

Docking

3-D QSAR

COMBINE


Select Reaction ×
Specify Reactants ×
Set Reactor Optio... ×
Run Reactor ×
Online Help
License Manager
Hide Sidebar
Scheme
Rules
Examples
Properties
Reactant standardization
Test
File
Edit
View
Insert
Atom
Bond
Structure
Calculations
Services
Help
97%
O
N
S
Fe
H
C
N
O
S
F
P
2D
Apply
Cancel
< Back
Next >
Finish
Cancel

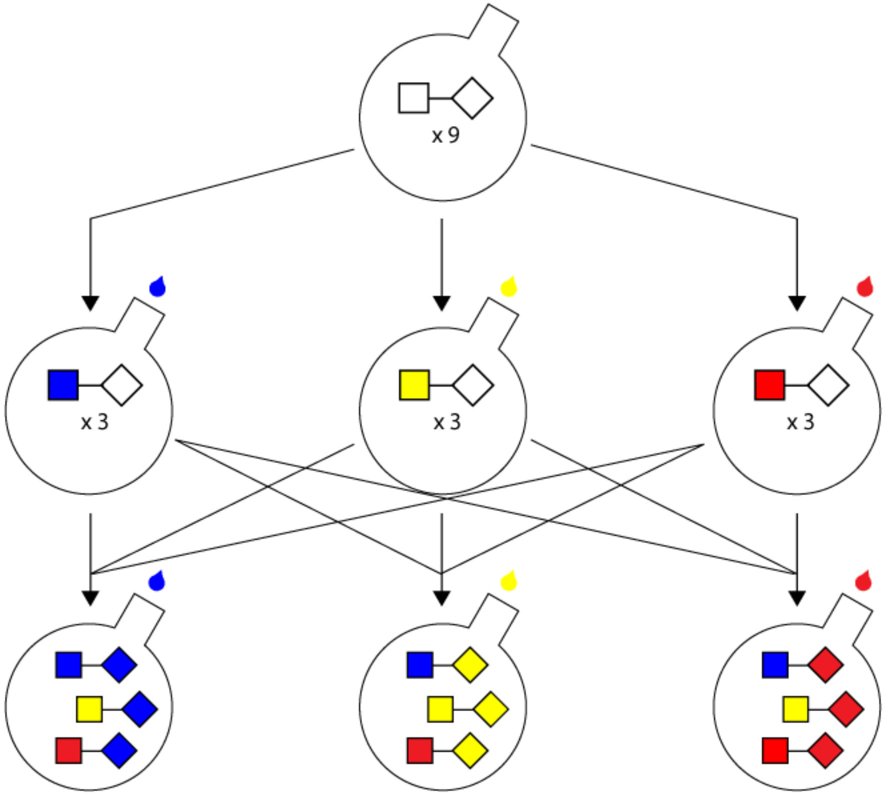
1. Unnamed

Select a reaction

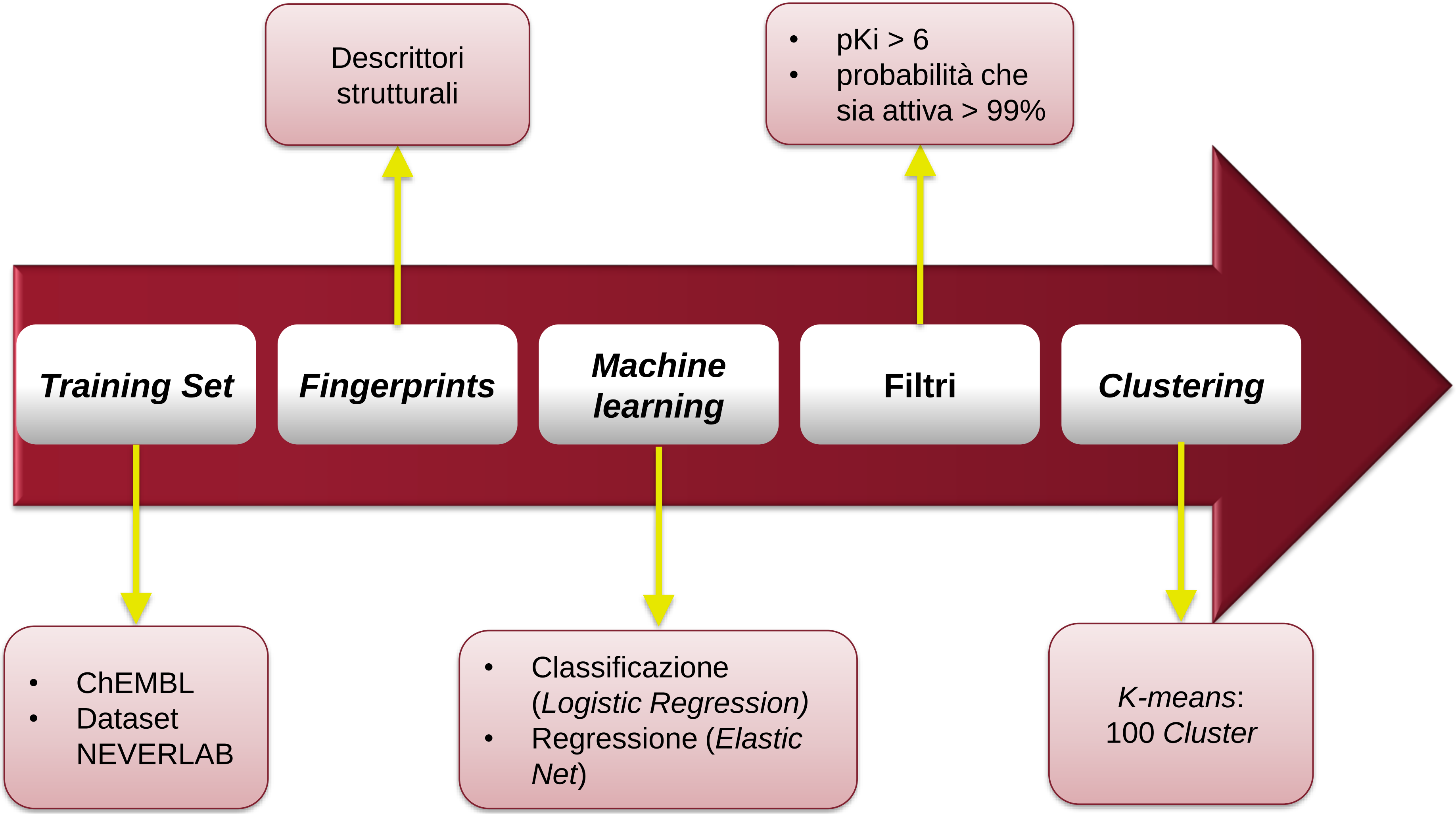
Reaction name: Unnamed

Press 'Next' to specify reactants

1,5 M molecole



QSAR



Descrittori strutturali

- pKi > 6
- probabilità che sia attiva > 99%

Training Set

Fingerprints

Machine learning

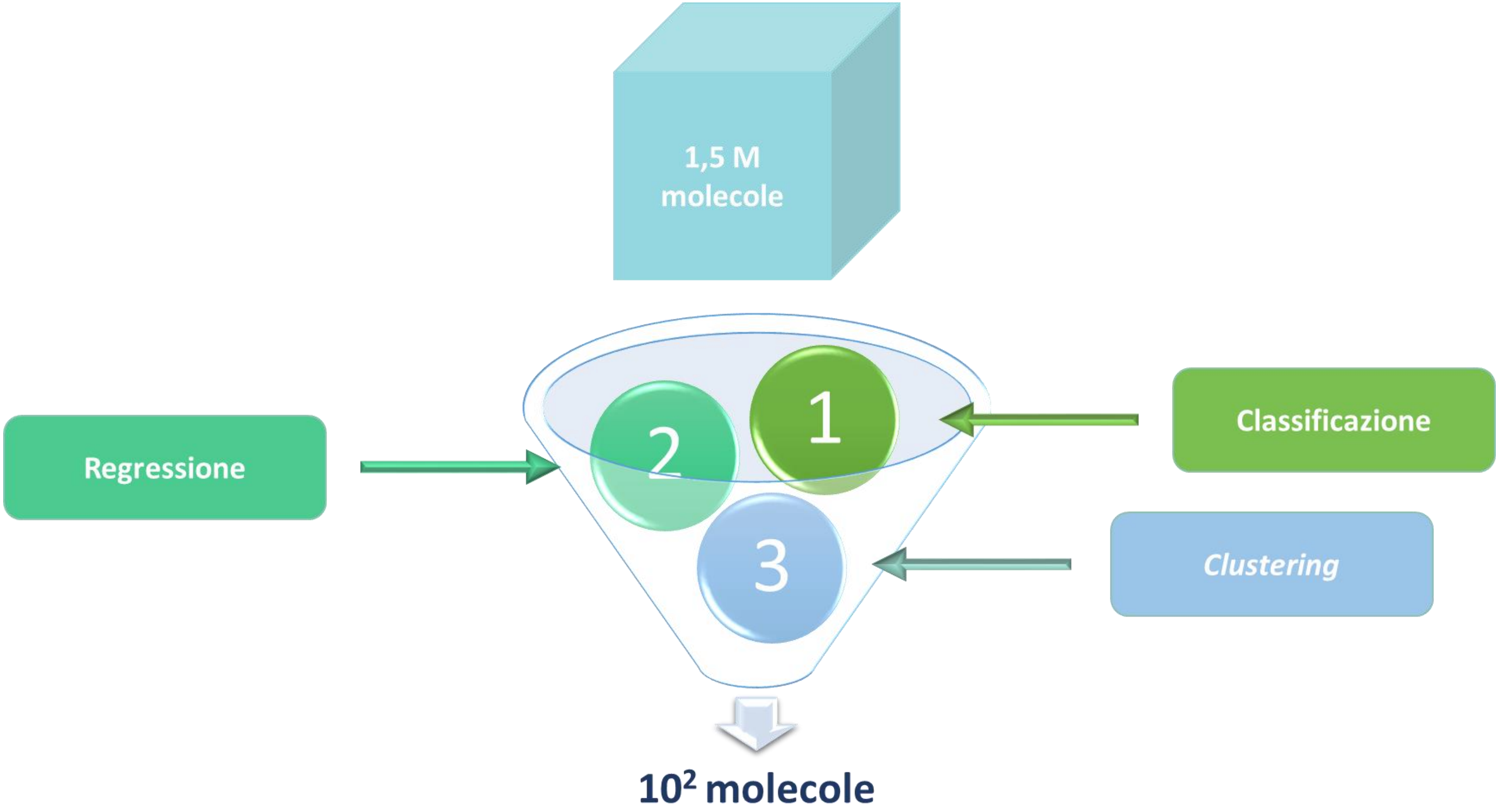
Filtri

Clustering

- ChEMBL
- Dataset NEVERLAB

- Classificazione (*Logistic Regression)*
- Regressione (*Elastic Net*)

K-means:
100 *Cluster*



1,5 M molecole

Regressione

Classificazione

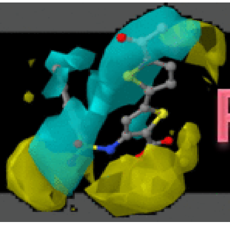
Clustering

1

2

3

10^2 molecole



PROCEDURA SPERIMENTALE/3

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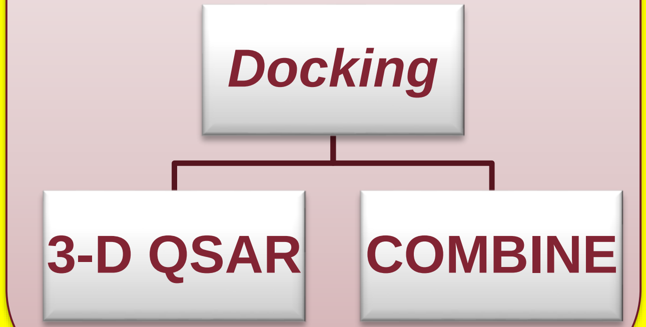
Parte sintetica

Sintesi molecole a struttura tiobarbiturica

Parte computazionale: Predizione

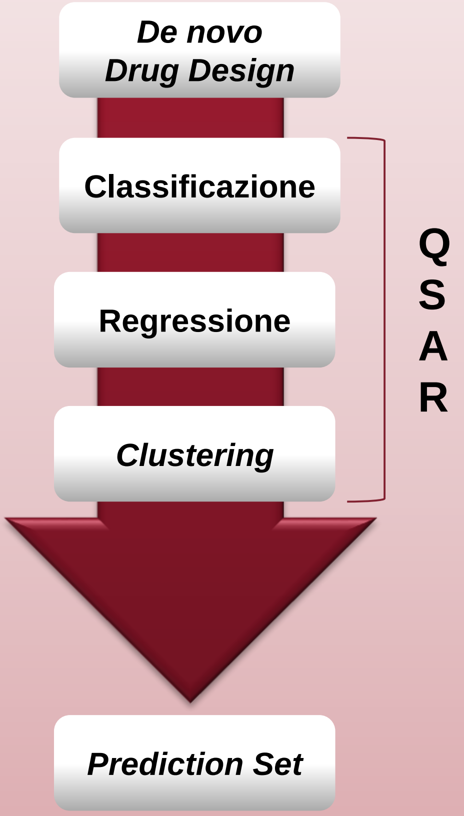


Docking

3-D QSAR

COMBINE

Parte computazionale: *Focus Virtual Screening*



De novo Drug Design

Classificazione

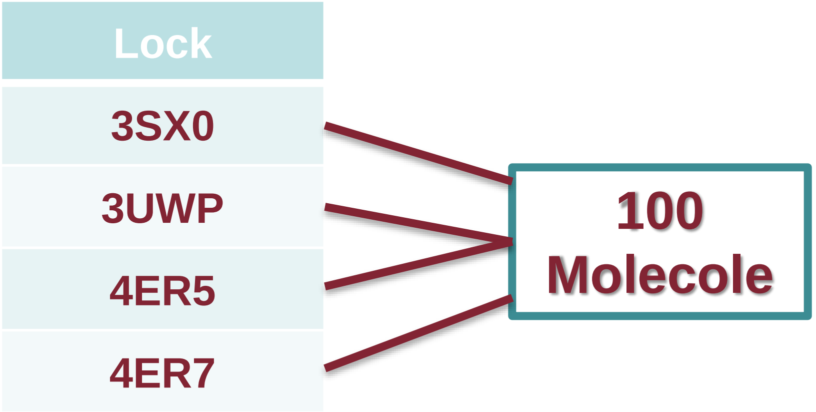
Regressione

Clustering

Prediction Set

QSAR

DOCKING



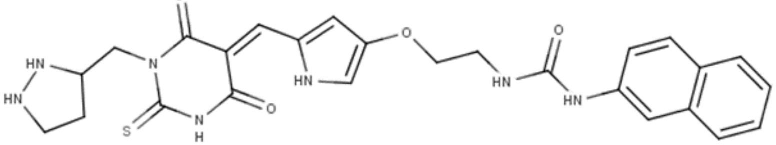
Lock
3SX0
3UWP
4ER5
4ER7

100 Molecole

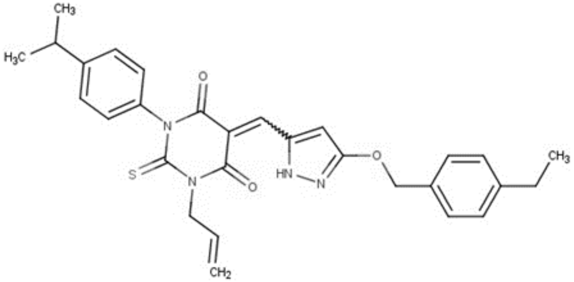
Surflex

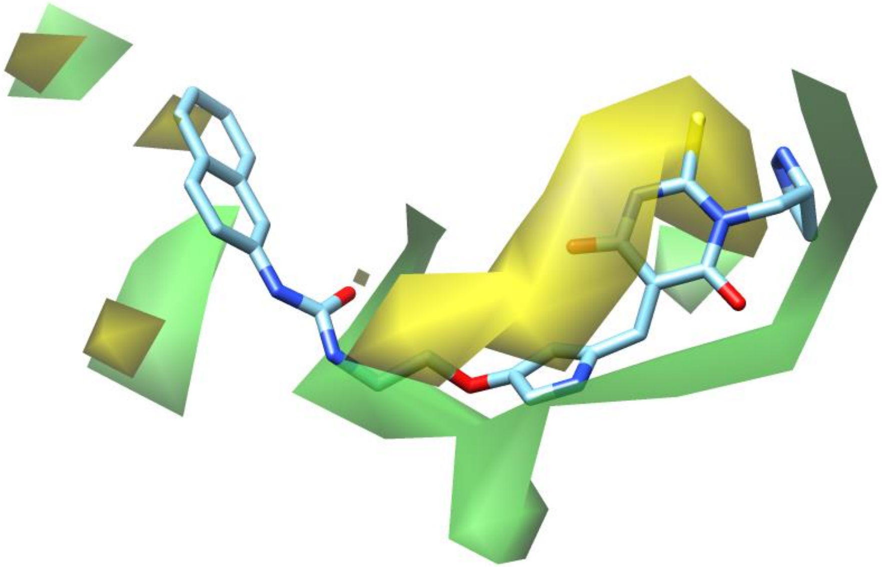
	Py-CoMFA Probe H	3-D QSAR PROBE HD
r^2	0,98	0,96
q^2	0,82	0,79
SDEP_pred	0,84	1,52
AAEP	1,12	1,22

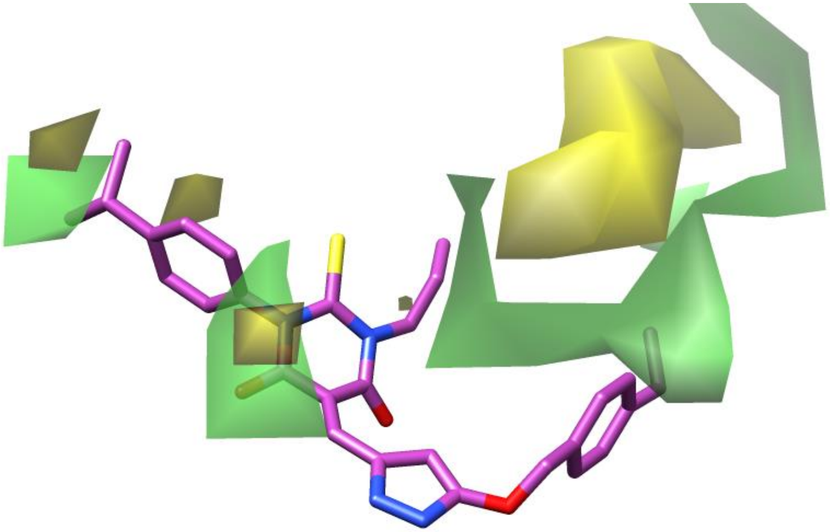
Molecola **più attiva**
1554915


HN
HN
N
O
S
N
H
O
HN
O
O
HN
HN

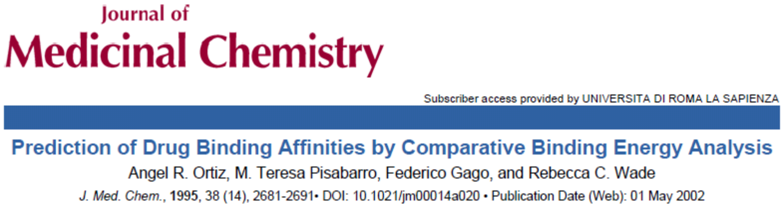
Molecola **meno attiva**
1342076


CH3
H3C
O
N
S
N
O
HN
N
O
CH3
CH2





COMBINE



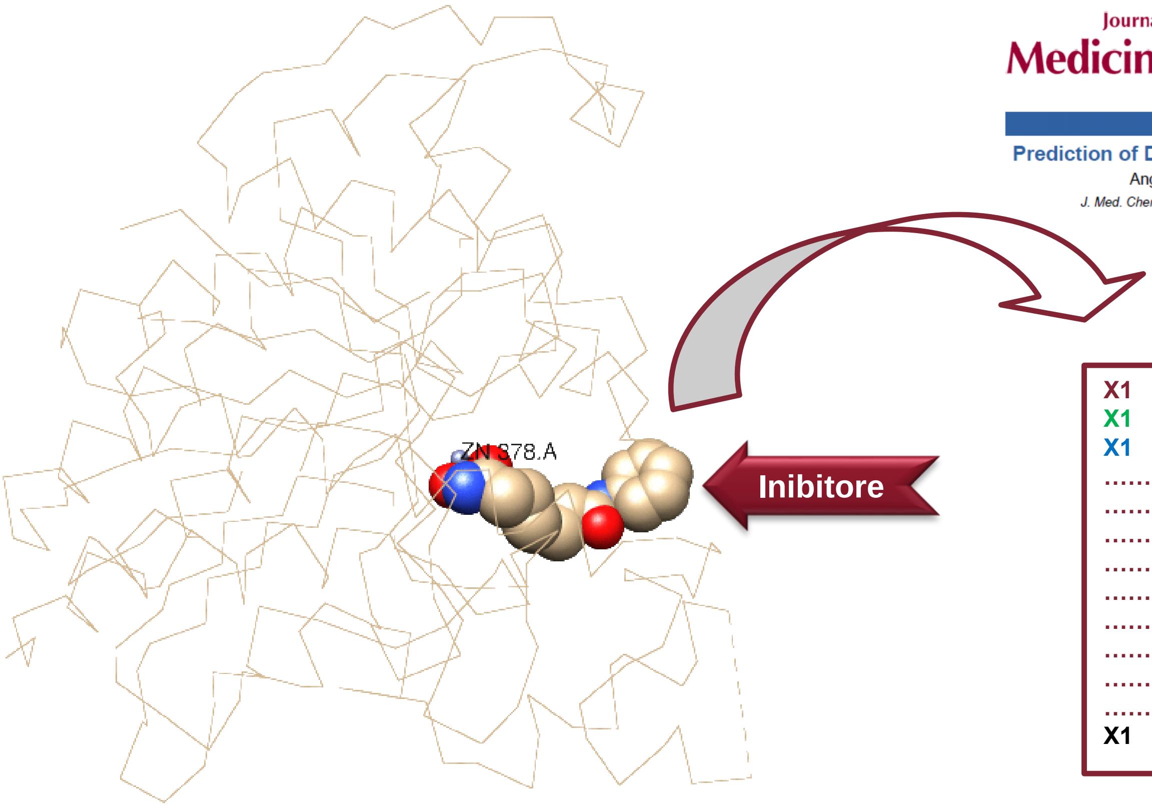
Journal of
Medicinal Chemistry

Subscriber access provided by UNIVERSITA DI ROMA LA SAPIENZA

Prediction of Drug Binding Affinities by Comparative Binding Energy Analysis

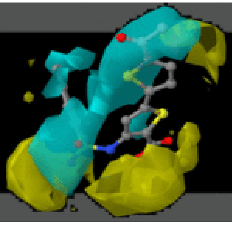
Angel R. Ortiz, M. Teresa Pisabarro, Federico Gago, and Rebecca C. Wade

J. Med. Chem., 1995, 38 (14), 2681-2691• DOI: 10.1021/jm00014a020 • Publication Date (Web): 01 May 2002



X1	X2	X3	....	Xn
X1	X2	X3	....	Xn
X1	X2	X3	....	Xn
....	....	....	....	....
....	....	....	....	....
....	....	....	....	....
....	....	....	....	....
....	....	....	....	....
....	....	....	....	....
....	....	....	....	....
....	....	....	....	....
....	....	....	....	....
X1	X2	X3	....	Xn

$$\Delta U = \sum_{i=1}^{n_l} \sum_{j=1}^{n_r} u_{ij}^{\mathrm{VDW}} + \sum_{i=1}^{n_l} \sum_{j=1}^{n_r} u_{ij}^{\mathrm{ELE}}$$



COMBINE

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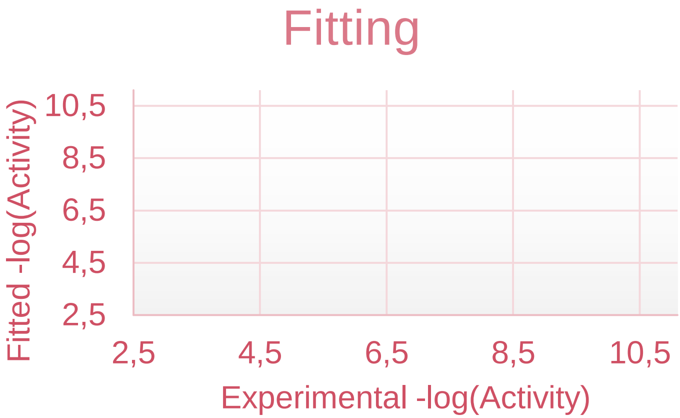
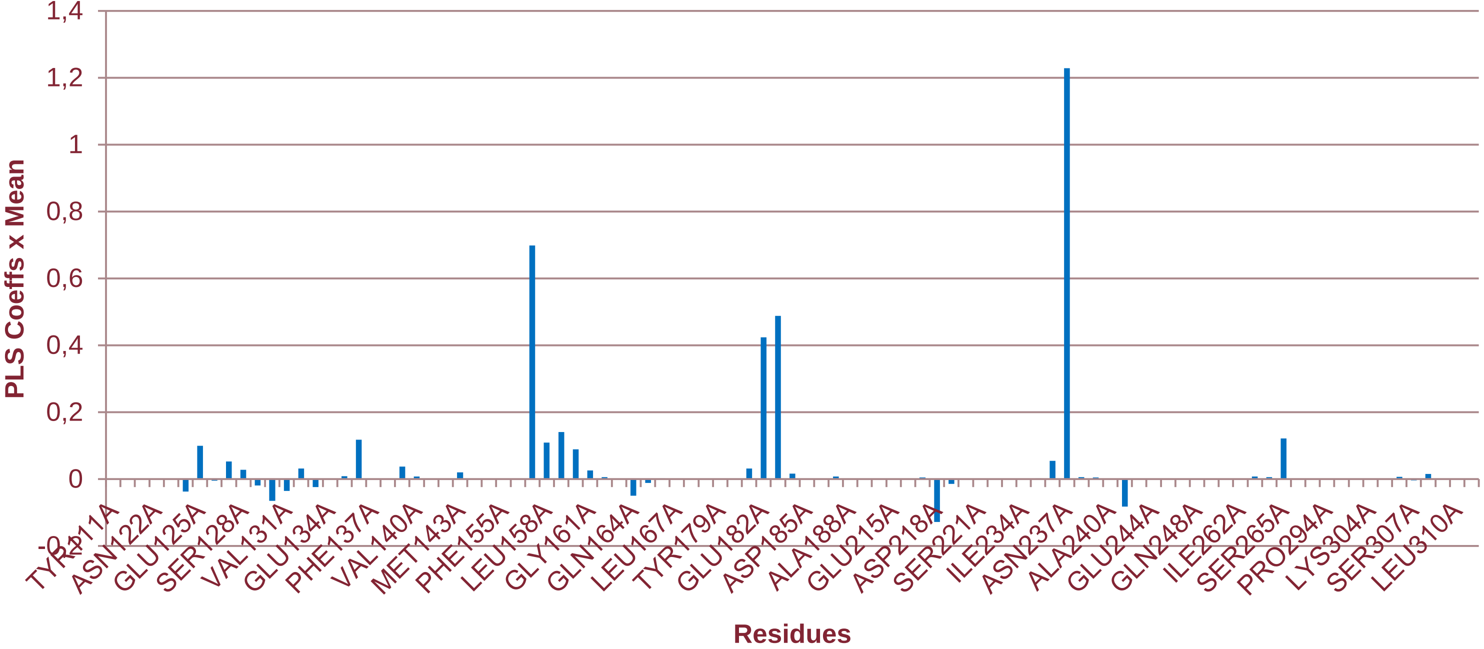
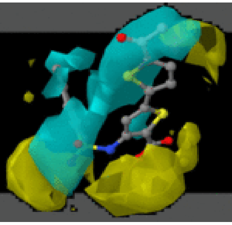
	COMBINE DRY.HB
***r*²**	0,92
***q*²**	0,69
SDEP_pred	1,36
AAEP	1,14







CONCLUSIONI

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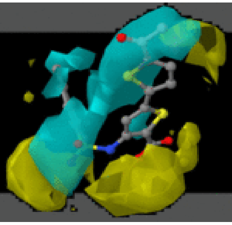
- Generazione e applicazione modelli 3-D QSAR, COMBINE
- Creazione nuova libreria di molecole
- Predizione attività nuove molecole
- Sintesi composti a struttura tiobarbiturica



OBIETTIVI FUTURI

- **Valutazione strategia sintetica delle molecole predette**
- **Utilizzo modelli per creazione ulteriori librerie**
- **Valutazione molecole tiobarbituriche sintetizzate attraverso saggi biochimici**

Grazie per l'attenzione