Laboratorio di chimica bioorganica 2021-22

L	11-ott	Berti lezione		18-ott	Berti sintesi	25-ott	Berti sintesi - lezione
M	12-ott	Pengo		19-ott	Pengo	26-ott	Berti cinetiche ed LC/MS. Mattina G1,G2. Pom. G3, G4.
M	13-ott	Pengo		20-ott	Pengo	27-ott	Berti cinetiche ed LC/MS. Mattina G5,G6. Pom. G7, G8.
G	14-ott	Pengo		21-ott	Pengo	28-ott	Berti cinetiche ed LC/MS. Mattina G9,G10. Pom. G11, G12.

Modalità di svolgimento dell'esame:

- Valutazione nel corso delle attività
- Presentazione orale (ppt) di una delle due esperienze
- Domande sull'altra esperienza

Laurea Magistrale in Chimica

Laboratorio di Chimica Bioorganica

anno accademico 2021 – 2022

Due librerie ortogonali di potenziali inibitori della tirosinasi

JOURNAL OF CHEMICAL EDUCATION

pubs.acs.org/jchemeduc Laboratory Experiment

Biginelli Reaction and β-Secretase Inhibition: A Multicomponent Reaction as a Friendly Educational Approach to Bioactive Compounds

Maria Funicello,* Iole Cerminara, Lucia Chiummiento, Paolo Lupattelli, Fulvia Felluga, and Federico Berti*

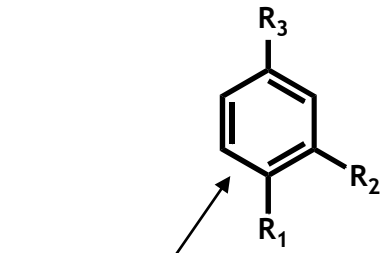
Cite This: *J. Chem. Educ.* 2021, 98, 1756–1761 Read Online

Introduzione alla chimica combinatoriale

$P_1—P_2—P_3$ 20^3 = 8000 tripeptidi

$P_1 \cdots P_{100}$ $20^{100} = 1 \times 10^{130}$ peptidi

Una libreria di origine biologica, ad es. fagica: $10^6 – 10^8$ membri

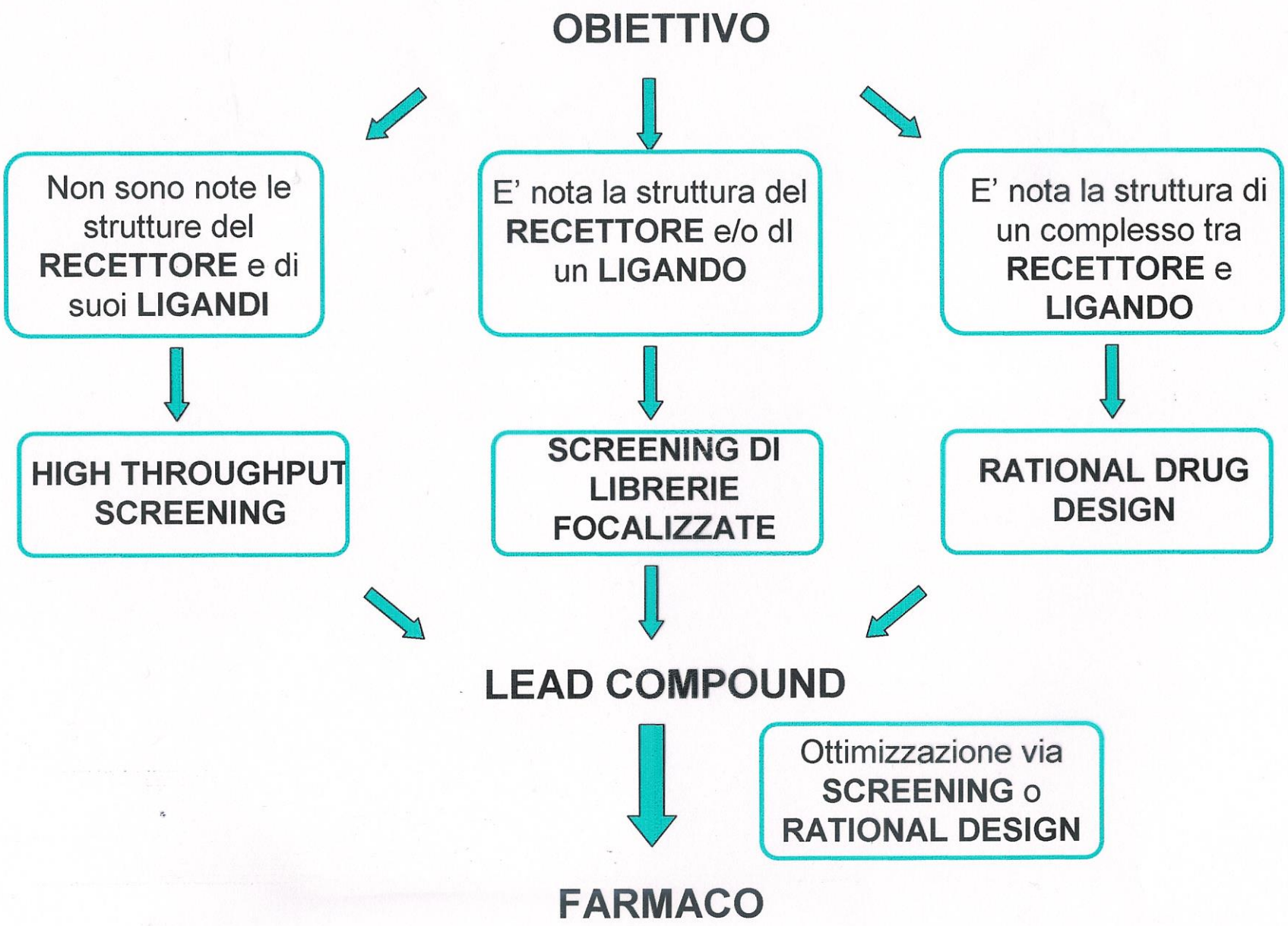


R_1	R_2	R_3
Me	F	OH
Et	Cl	NH_2
Pr	Br	SH
i-Pr	I	$NHCOCH_3$

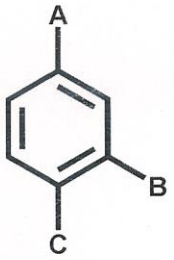
64 composti: una libreria di sintesi

Scheletro (scaffold) non polimerico

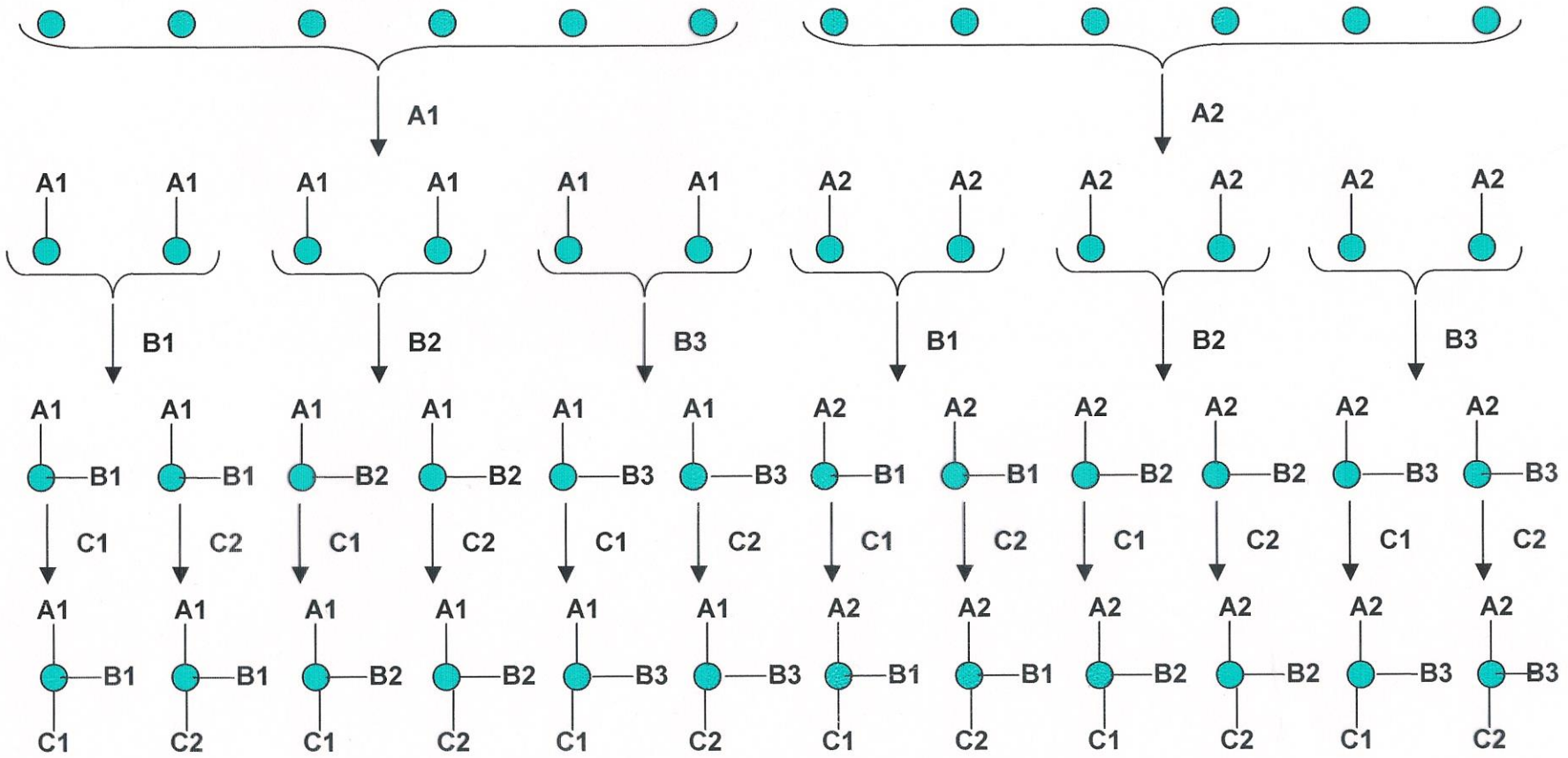
gruppi funzionali


OBIETTIVO
Non sono note le strutture del RECETTORE e di suoi LIGANDI
E' nota la struttura del RECETTORE e/o dl un LIGANDO
E' nota la struttura di un complesso tra RECETTORE e LIGANDO
HIGH THROUGHPUT SCREENING
SCREENING DI LIBRERIE FOCALIZZATE
RATIONAL DRUG DESIGN
LEAD COMPOUND
Ottimizzazione via SCREENING o RATIONAL DESIGN
FARMACO

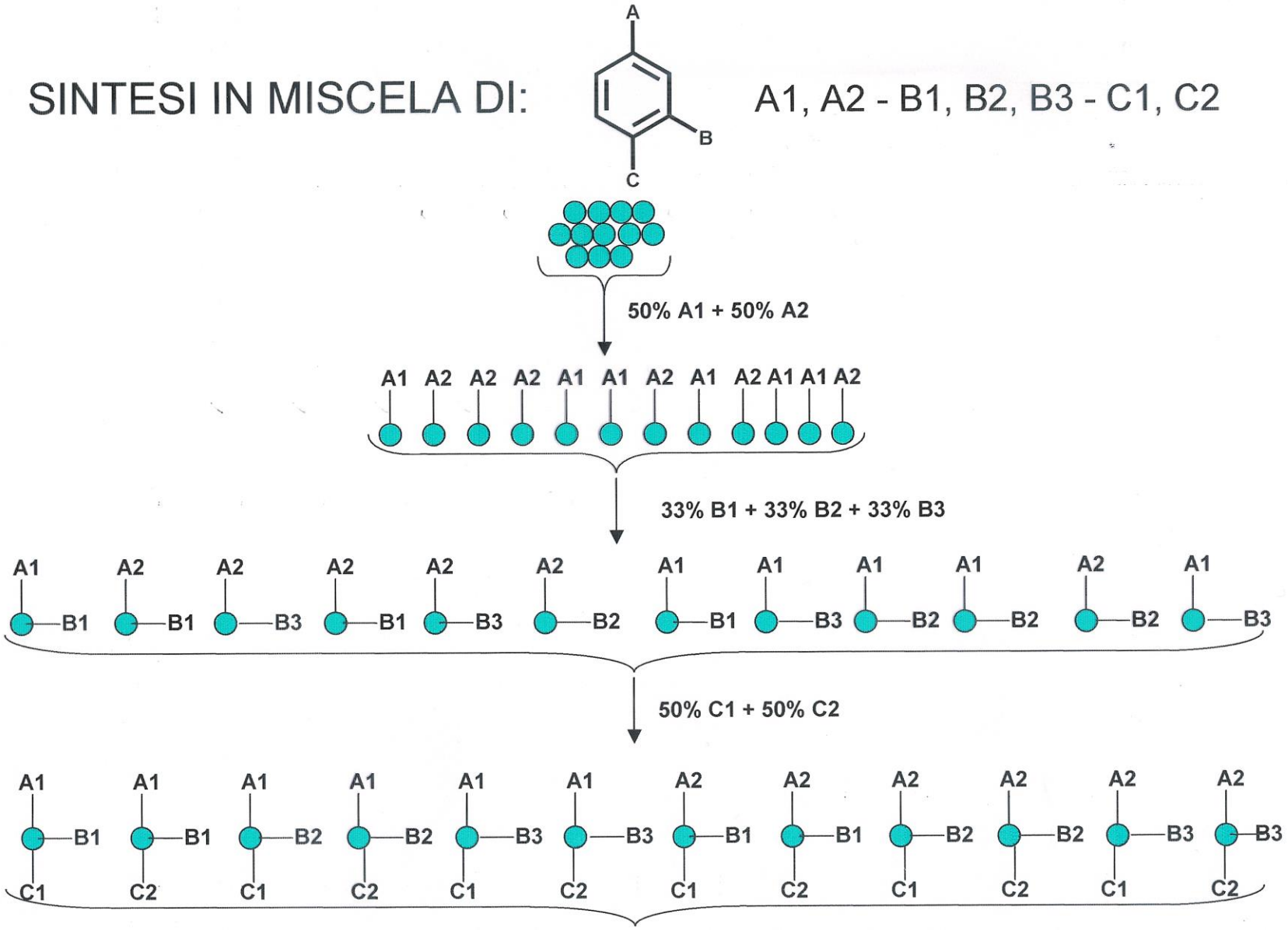
SINTESI PARALLELA DI:



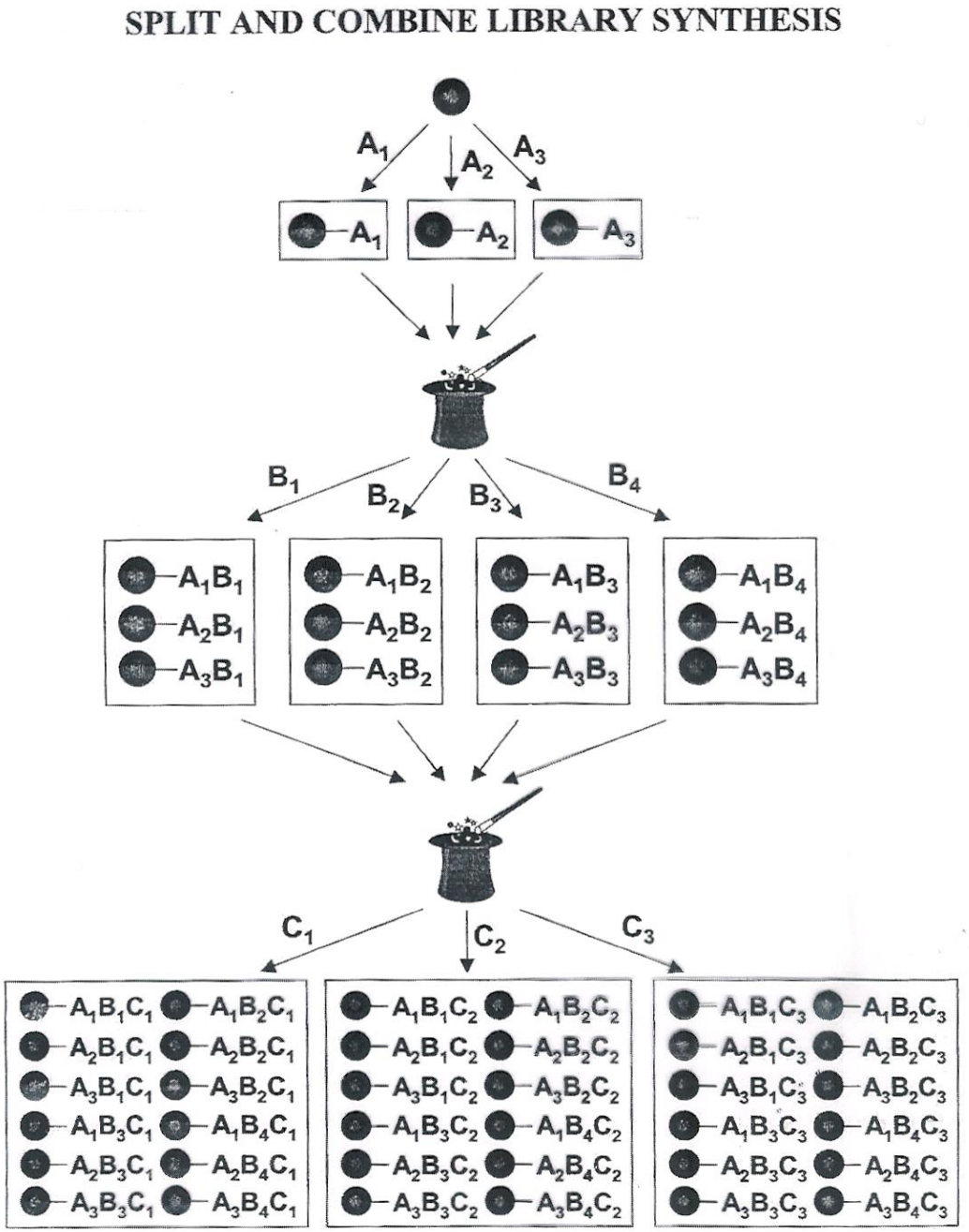
A1, A2 - B1, B2, B3 - C1, C2



2+6+12=20 reazioni per 12 composti

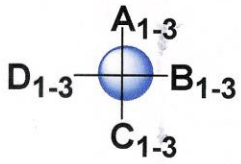


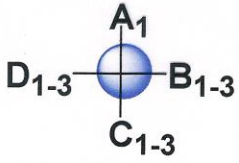
Tre reazioni per una libreria di 12 composti

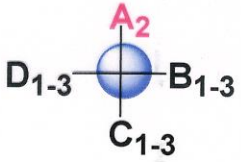


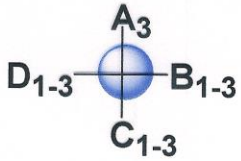
Sintesi in miscela di una libreria composta di una gerarchia di sottolibrerie

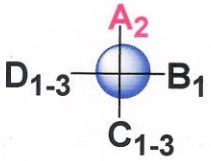
DECONVOLUZIONE ITERATIVA

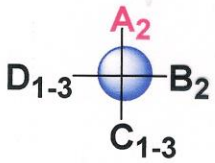


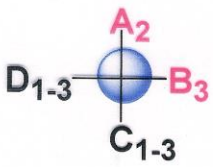


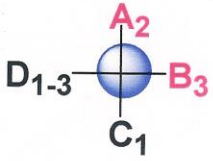


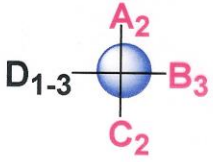


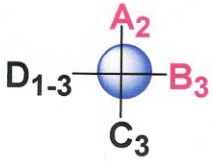


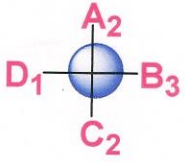


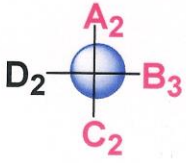


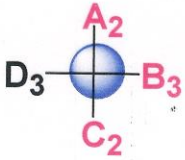




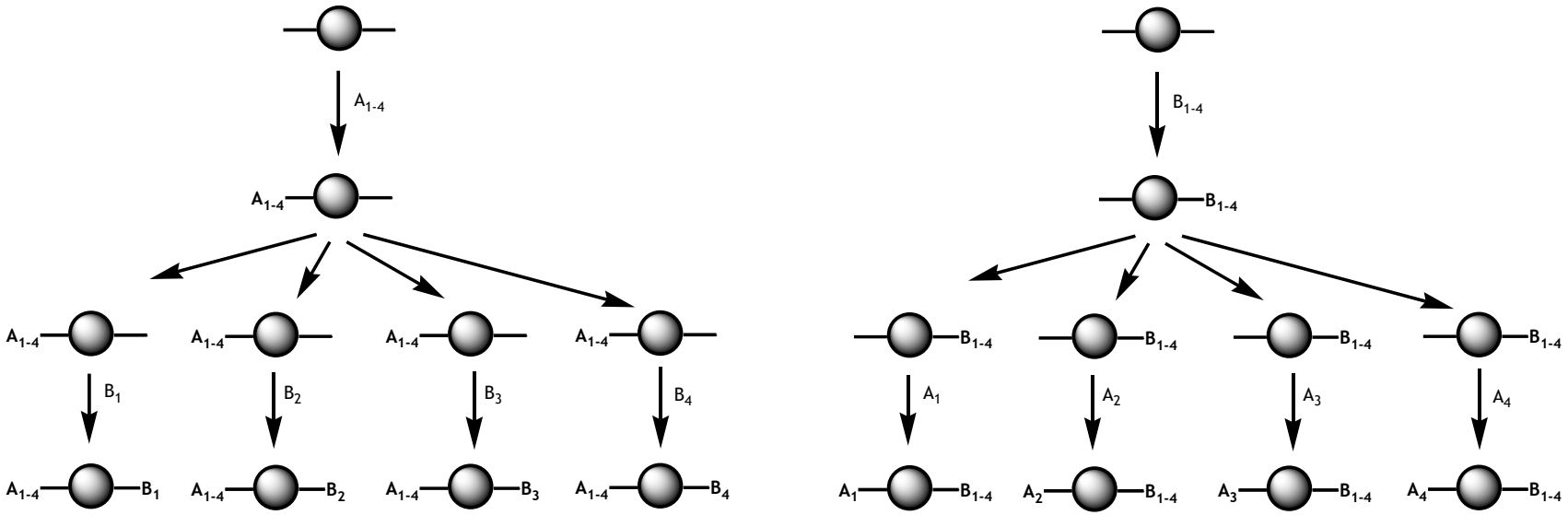






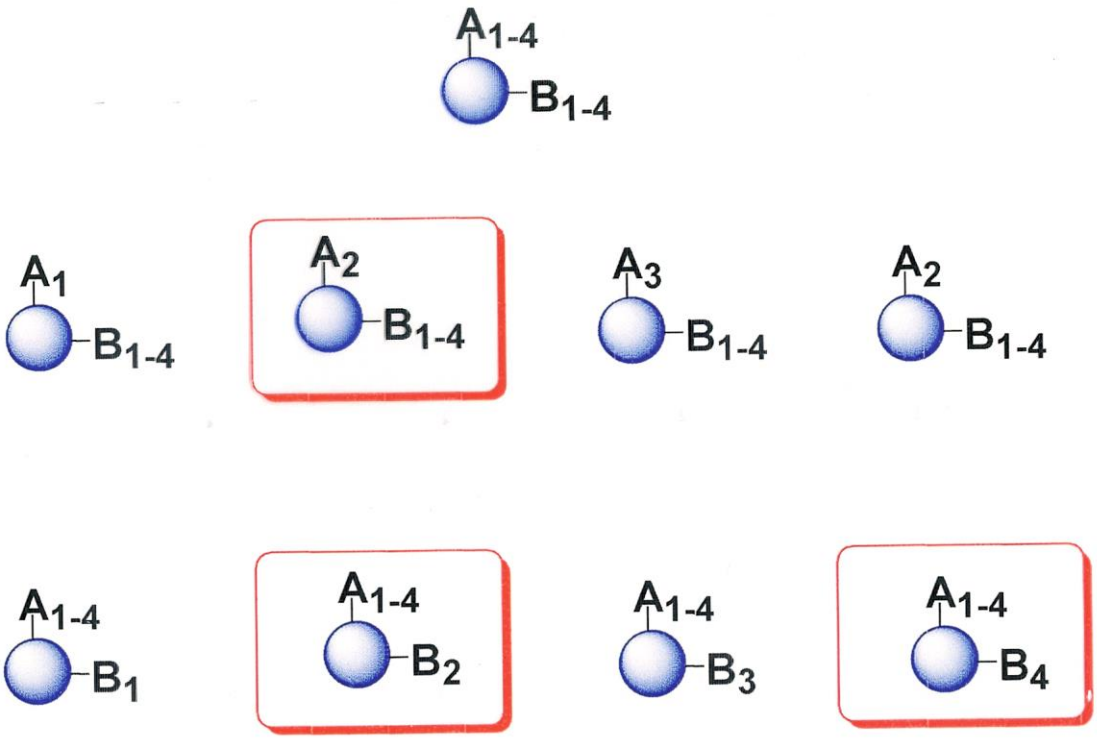


Sintesi in miscela di librerie ortogonali



100 A, 100 B: 10000 composti, 10000 reazioni per la sintesi parallela pura. 202 reazioni in quattro passaggi per la sintesi di librerie ortogonali.

Deconvoluzione di librerie ortogonali



Attivi: A_2B_2 e A_2B_4

La melanogenesi

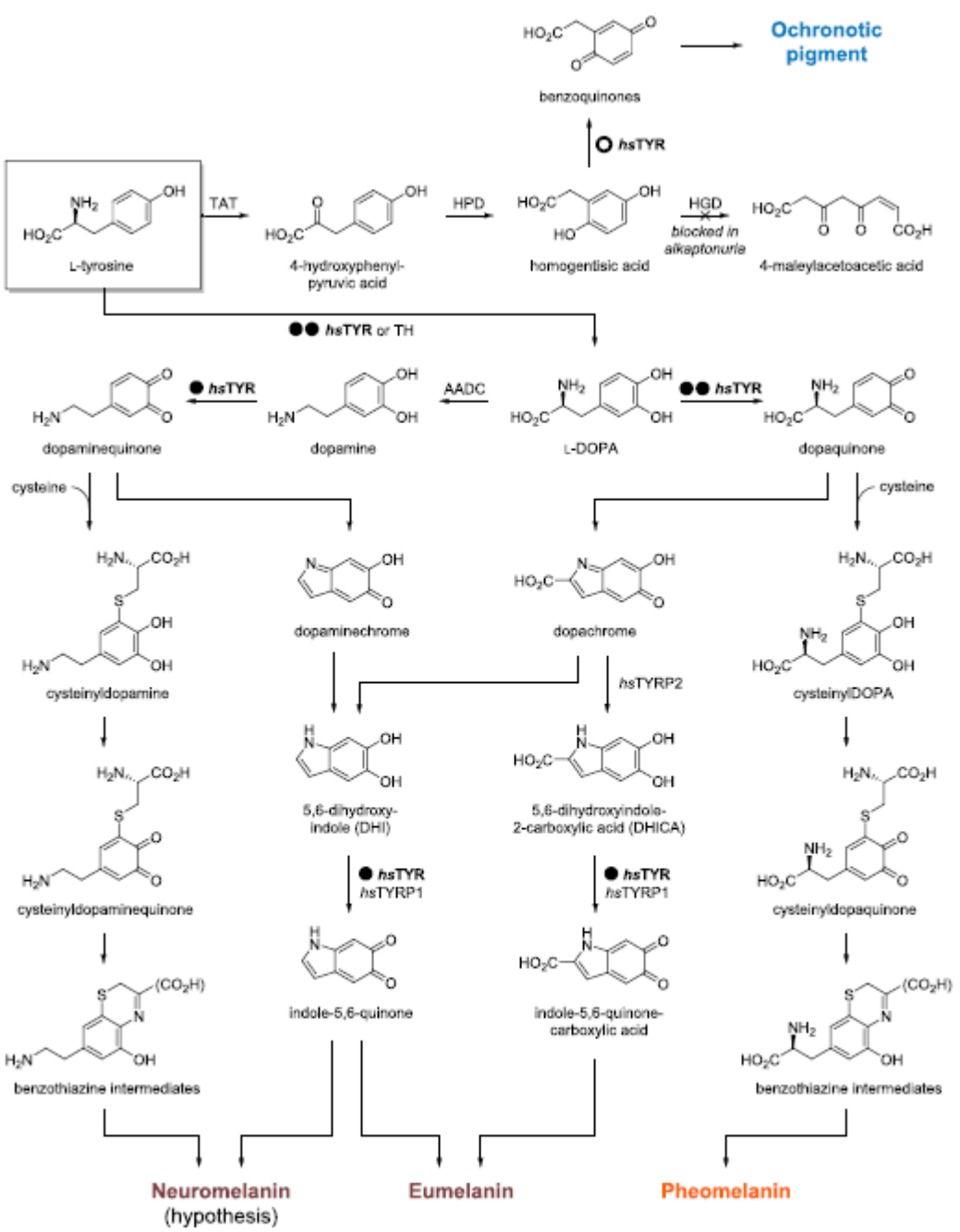
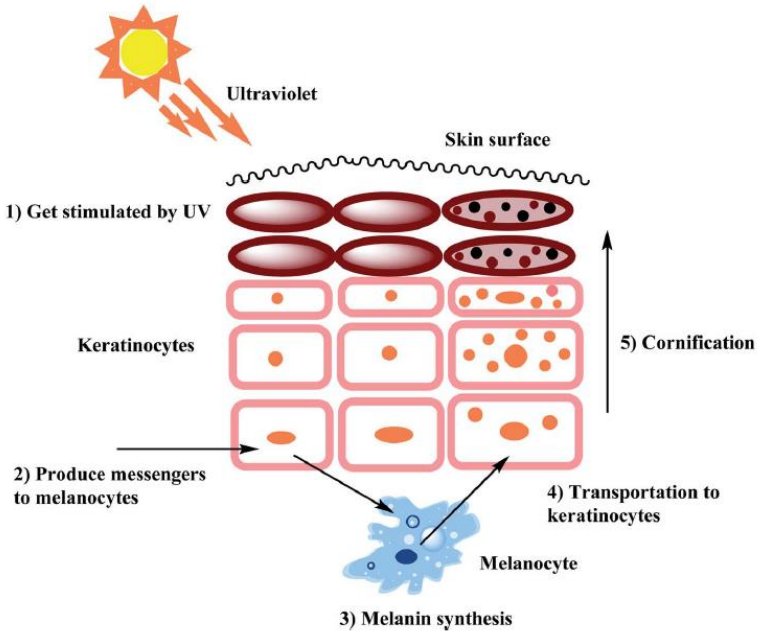
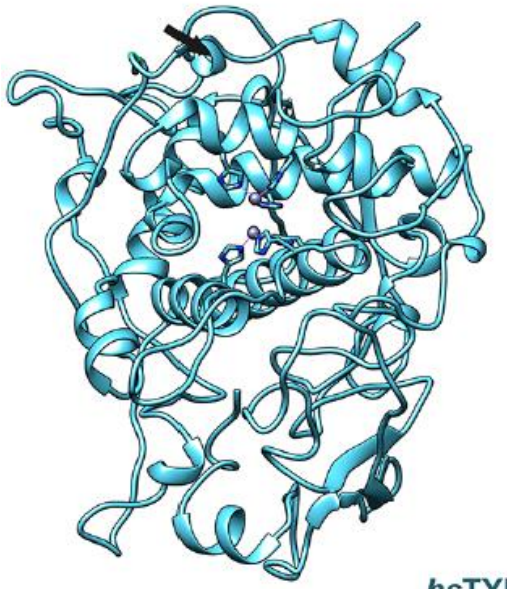


Figure 1. L-Tyrosine-related pigmentation biochemical pathways involving *hs*TYR as a demonstrated (●●), suspected (●) or purely hypothetic (○) component.

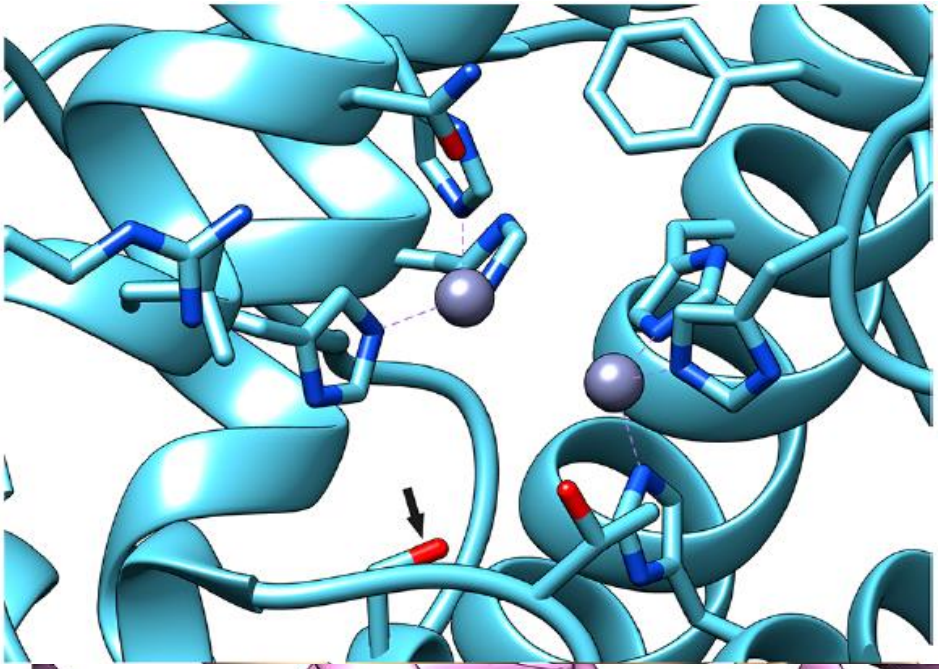


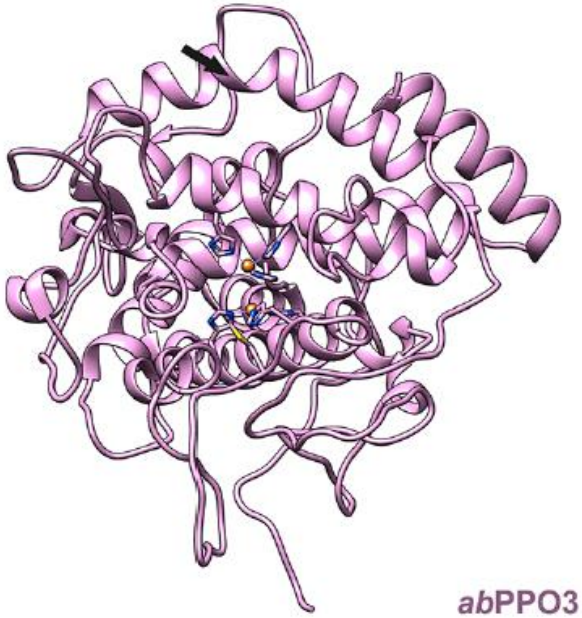
Inibizione della tirosinasi:

- Skin whitening
- Trattamenti dermatologici (melasma, lentigo solare…)
- Terapia del melanoma
- Parkinson (neuromelanina)
- Ocronosi e alcaptonuria (accumulo di acido omogentisico)

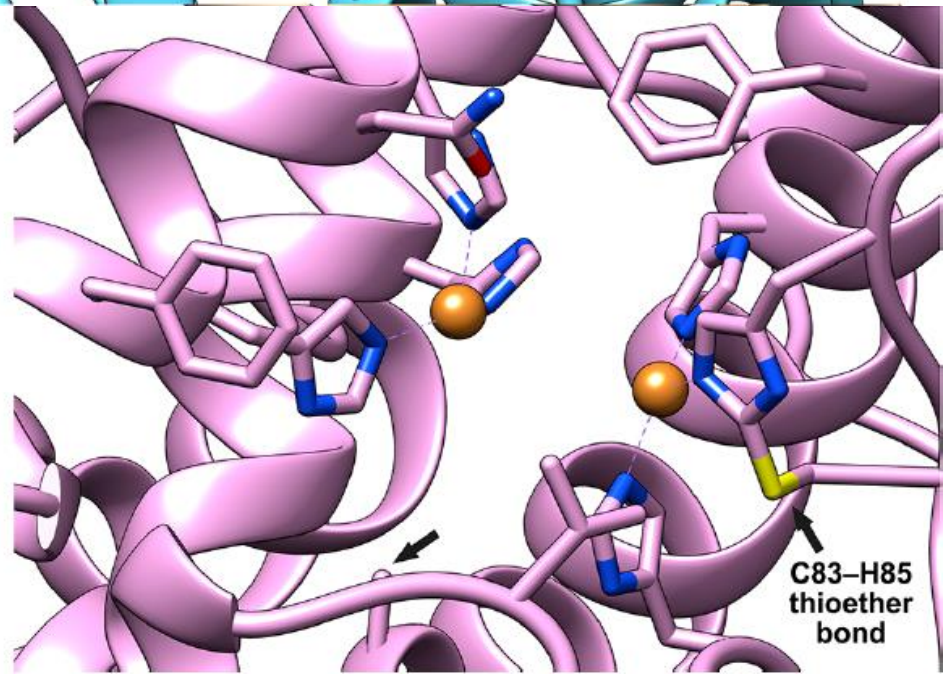


*hs*TYRP1





*ab*PPO3



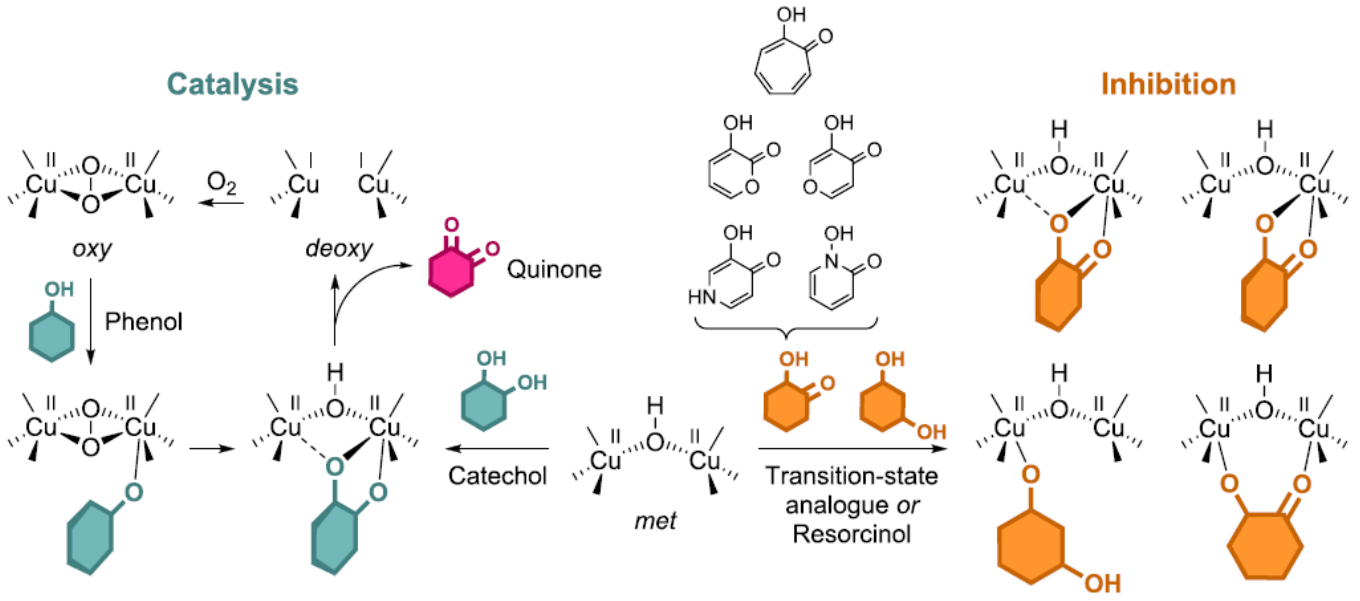
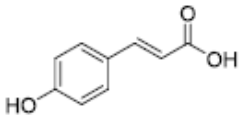
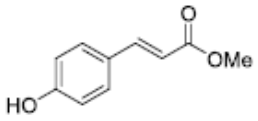


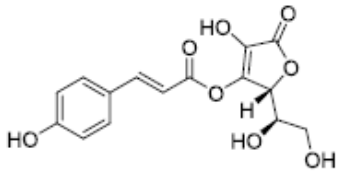
Figure 6. Simplified catalytic cycle of the oxidation of 4-phenols and 3,4-catechols by TYRs, and examples of possible binding modes for nonoxidizable transition-state analogues and resorcinols as active-site inhibitors.



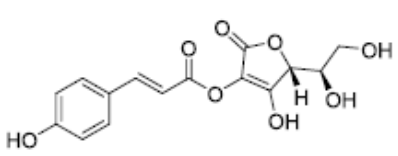
1
p-Coumaric acid
IC_{50} = 2–6 μM
(HEK293-TYR, HEMn)



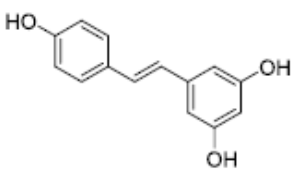
2
p-Coumaric acid methyl ester
IC_{50} = 30 μM
(HEMn)



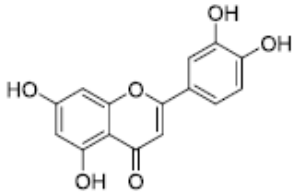
3
Ascorbyl 3-*p*-coumarate
IC_{50} = 10 μM
(HEK293-TYR)



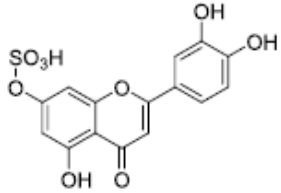
4
Ascorbyl 2-*p*-coumarate
IC_{50} = 40 μM
(HEK293-TYR)



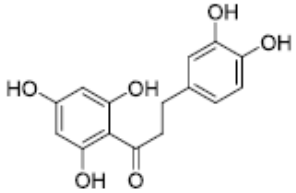
5
Resveratrol
IC_{50} = 1.7–9 μM
(HEK293-TYR)



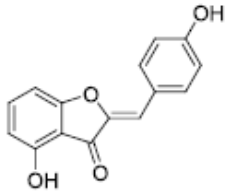
6
Luteolin
IC_{50} = 60 μM
(HEK293-TYR)



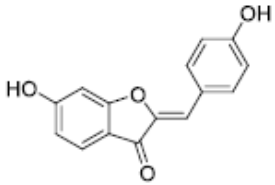
7
Luteolin-7-sulfate
IC_{50} = 16 μM
(HEK293-TYR)



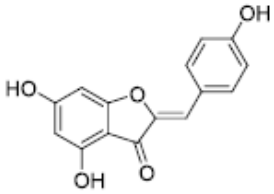
8
3-Hydroxyphloretin
IC_{50} = 32 μM
(HEMn)



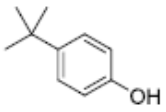
9
6,4'-Dihydroxyaurone
IC_{50} = 32 μM
(HEMa)



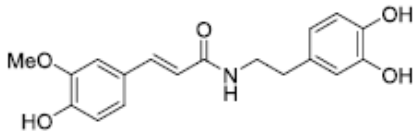
10
Hispidol
IC_{50} = 38 μM
(HEMa)



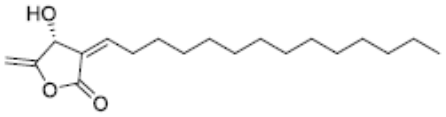
11
4,6,4'-Trihydroxyaurone
IC_{50} = 38 μM
(HEMa)



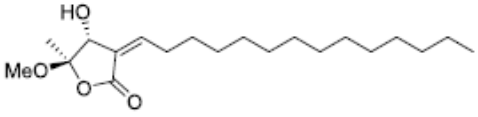
12
4-*t*-Butylphenol
IC_{50} = 10 μM
(HEMn)



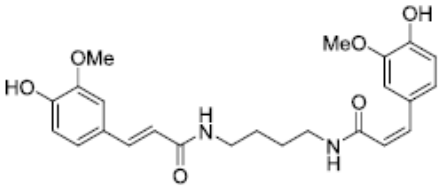
13
N-feruloyldopamine
IC_{50} = 50 μM
(HEMn)



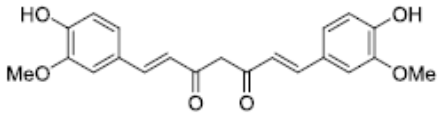
14
Linderanolide B
IC_{50} = 1 μM
(HEMn)



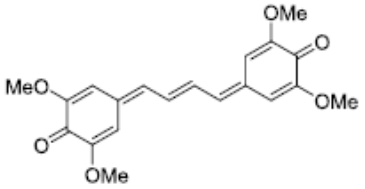
15
Subamolide A
IC_{50} = 1 μM
(HEMn)



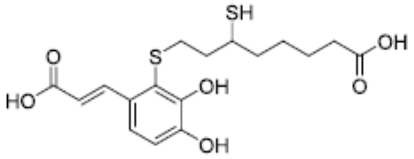
16
N-feruloyl-*N'*-*cis*-feruloylputrescine
IC_{50} = 85 μM
(HEMn)



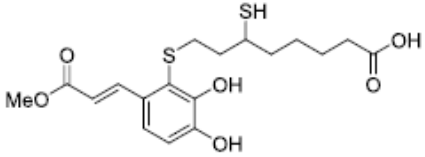
17
Curcumin
IC_{50} = 5 μM
(HEMa)



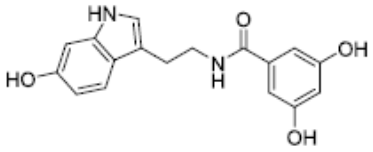
18
Kinobeon A
IC_{50} = 2.5 μM
(HMV-II)



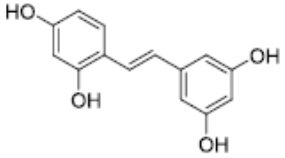
19
2-S-Lipoylcaffeic acid
IC_{50} = 76 μM
(HBL)



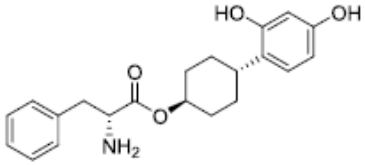
20
2-S-Lipoylcaffeic acid methyl ester
IC_{50} = 30 μM
(HBL)



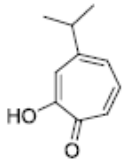
21
N-(3,5-Dihydroxybenzoyl)-6-hydroxytryptamine
IC_{50} = 9.1 μM
(HMV-II)



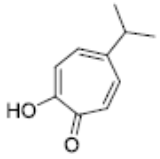
22
Oxyresveratrol
IC_{50} = 0.09–7.6 μM
(HEK293-TYR, HMV-II)



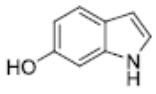
23
trans-4-(2,4-Dihydroxyphenyl)cyclohexyl-(2*R*)-2-amino-3-phenylpropanoate
IC_{50} = 2 μM
(SKMEL)



24
β-Thujaplicin
IC_{50} = 9 μM
(G-361)

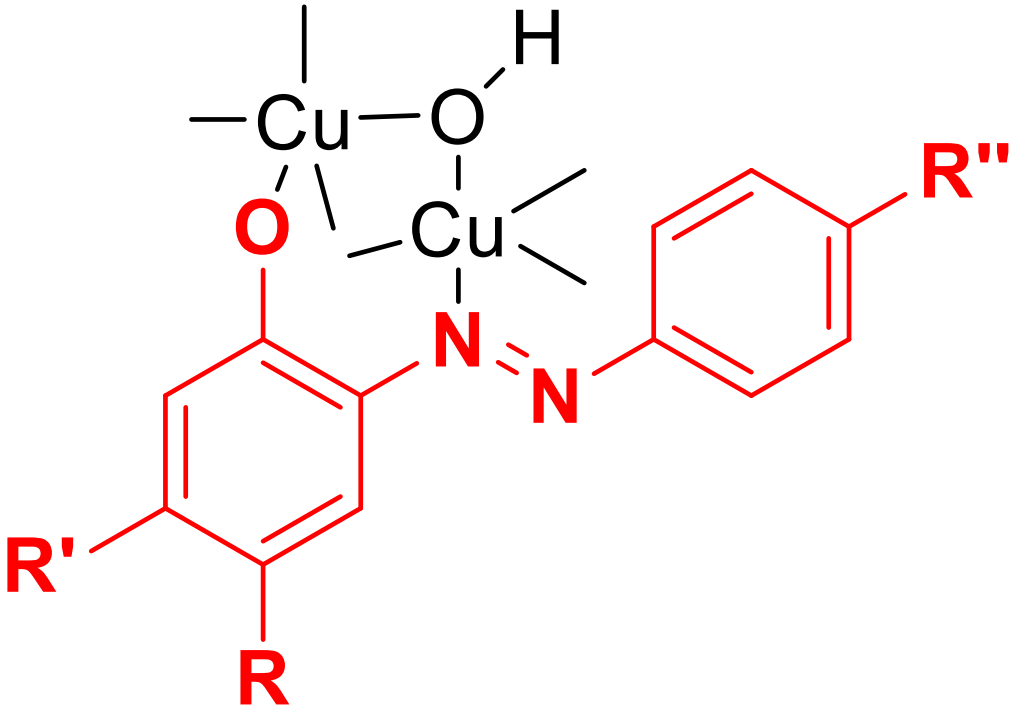


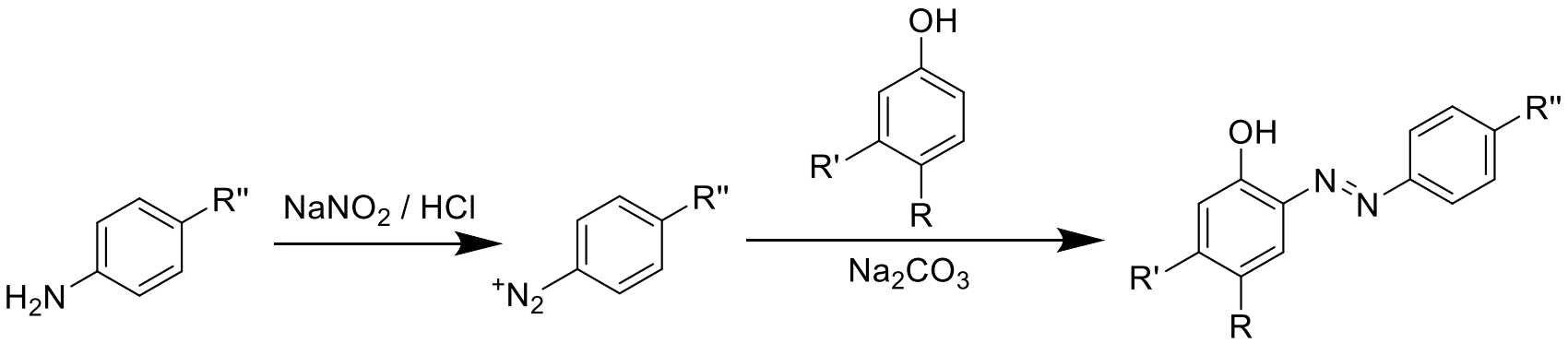
25
γ-Thujaplicin
IC_{50} = 1.2 μM
(G-361)

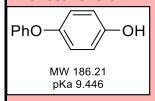
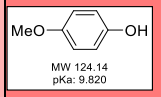
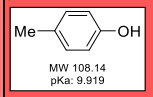
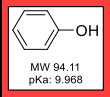
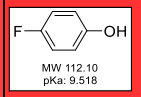
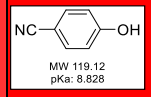
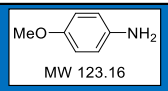
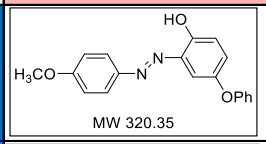
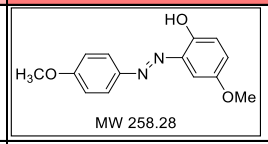
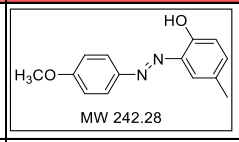
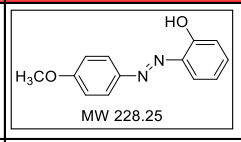
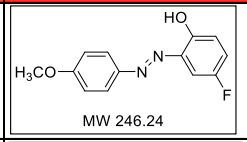
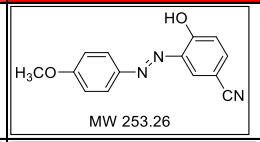
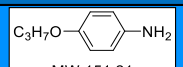
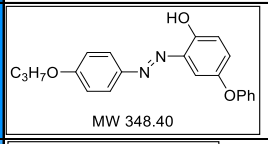
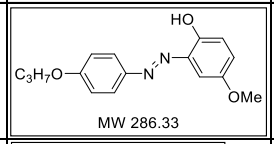
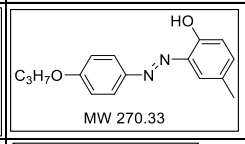
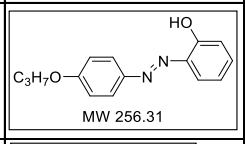
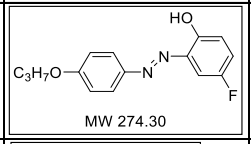
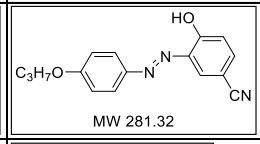
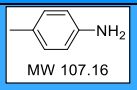
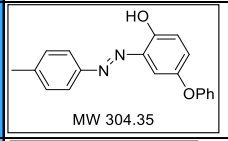
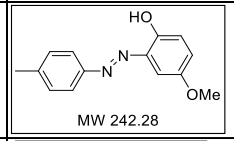
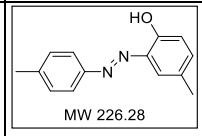
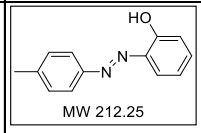
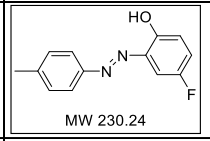
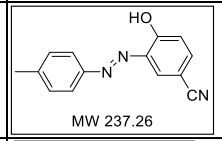
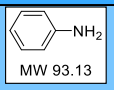
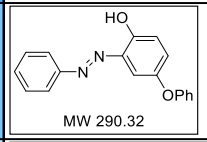
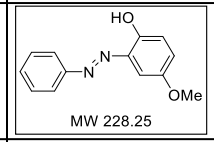
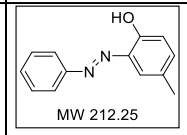
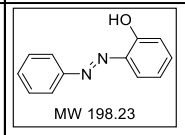
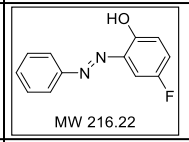
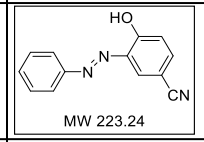
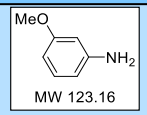
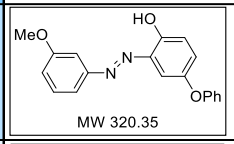
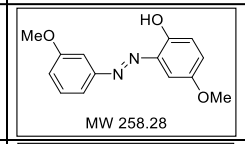
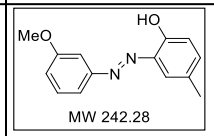
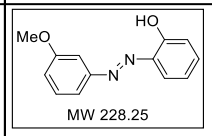
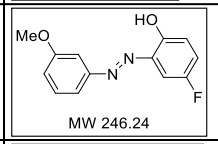
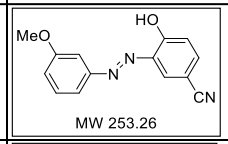
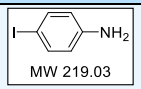
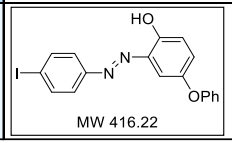
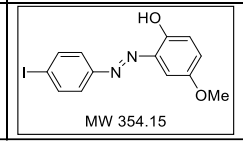
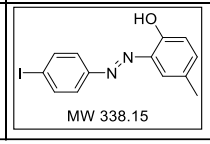
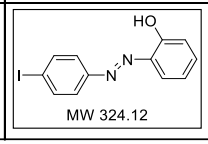
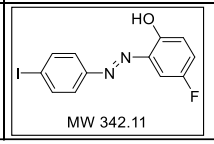
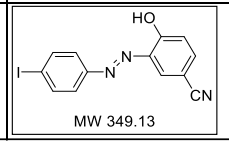


26
6-Hydroxyindole
IC_{50} = 22 μM
(HMV-II)

Il nostro target




R''
H2N
NaNO2 / HCl
R''
+N2
OH
R'
R
Na2CO3
OH
N=N
R''
R'
R

		σ	**F1** 4-fenossifenolo  MW 186.21 pKa 9.446 -0.35	**F2** 4-metossifenolo  MW 124.14 pKa: 9.820 -0.27	**F3** p-cresolo  MW 108.14 pKa: 9.919 -0.17	**F4** fenolo  MW 94.11 pKa: 9.968 0	**F5** 4-fluorofenolo  MW 112.10 pKa: 9.518 0.06	**F6** 4-cianofenolo  MW 119.12 pKa: 8.828 0.66
p-anisidina **A1**	 MW 123.16	-0.27	 MW 320.35	 MW 258.28	 MW 242.28	 MW 228.25	 MW 246.24	 MW 253.26
4-propossianilina **A2**	 MW 151.21	-0.24	 MW 348.40	 MW 286.33	 MW 270.33	 MW 256.31	 MW 274.30	 MW 281.32
p-toluidina **A3**	 MW 107.16	-0.17	 MW 304.35	 MW 242.28	 MW 226.28	 MW 212.25	 MW 230.24	 MW 237.26
anilina **A4**	 MW 93.13	0	 MW 290.32	 MW 228.25	 MW 212.25	 MW 198.23	 MW 216.22	 MW 223.24
m-anisidina **A5**	 MW 123.16	0.12	 MW 320.35	 MW 258.28	 MW 242.28	 MW 228.25	 MW 246.24	 MW 253.26
4-iodoanilina **A6**	 MW 219.03	0.18	 MW 416.22	 MW 354.15	 MW 338.15	 MW 324.12	 MW 342.11	 MW 349.13

Le reazioni sono condotte su una scala di 100 μmol di ciascun composto.

Librerie A – risolte in anilina

600 μmol di una anilina per ciascun gruppo vengono sciolte/sospese in 20 mL di acido cloridrico 0.1 M. La soluzione viene raffreddata a 0°C e si aggiungono lentamente 720 μmol di sodio nitrito in 4 mL di acqua. Si lascia reagire a 0°C per 15 minuti.
100 μmol di ciascun fenolo vengono sciolte/sospese in miscela in 20 mL di sodio carbonato 0.1 M. La soluzione viene raffreddata in acqua e aggiunta lentamente alla soluzione dell'anilina. Si lascia reagire per 30' a 0°C e si lascia poi salire la temperatura fino a RT.

Librerie F – risolte in fenolo

600 μmol di ciascuna anilina vengono sciolte/sospese in miscela in 20 mL di acido cloridrico 0.1 M. La soluzione viene raffreddata a 0°C e si aggiungono lentamente 720 μmol di sodio nitrito in 4 mL di acqua. Si lascia reagire a 0°C per 15 minuti.
100 μmol di un fenolo per ciascun gruppo vengono sciolte/sospese in miscela in 20 mL di sodio carbonato 0.1 M. La soluzione viene raffreddata in acqua e aggiunta lentamente alla soluzione dell'anilina. Si lascia reagire per 30' a 0°C e si lascia poi salire la temperatura fino a RT.

In entrambi i casi i grezzi di reazione vengono estratti con diclorometano. Le fasi organiche raccolte vengono seccate su sodio solfato anidro, ed il solvente viene evaporato a pressione ridotta in un pallone pesato. Il residuo, pesato, viene ridisciolto in un volume di DMSO tale da ottenere una concentrazione teorica 4 mM di ciascun composto, ipotizzando un peso molecolare medio di 277 per i composti della libreria (madre 80x).