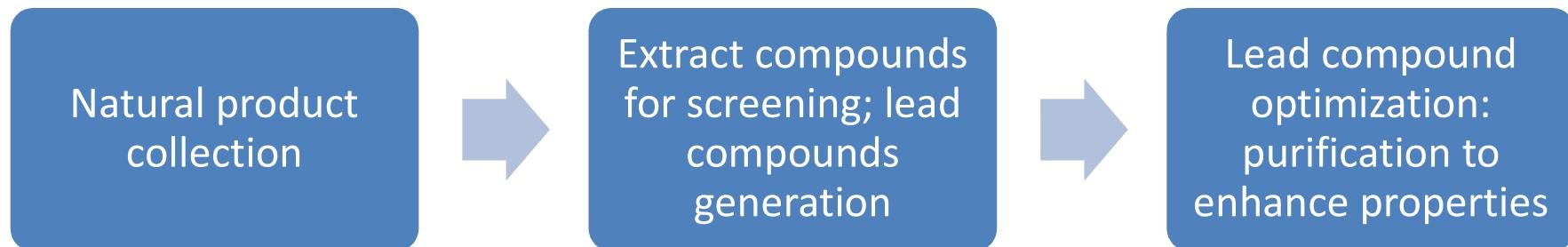


Desenho de fármacos: racional *versus* “irracional”

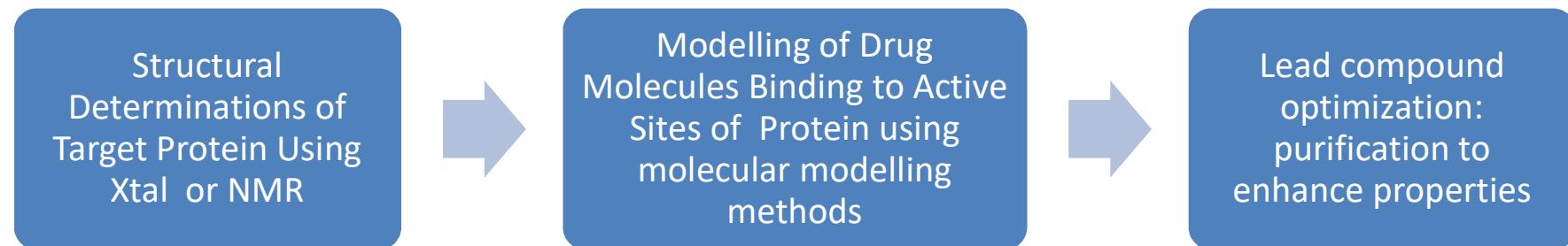
- Abordagem tradicional: empiricismo, intuição, medicina tradicional, pesquisa de força bruta (High-throughput screening)
- Desenho racional: busca de moléculas com características físico-químicas adequadas à actuação sobre o mecanismo da doença e à eficiente absorção/metabolização/eliminação

Irrational drug design approach



Example: One of the first angiotensin converting enzyme (ACE) inhibitors was teprotide. It is an antihypertensive drug for use after heart attacks. The active ingredient was isolated from the venom of a South American viper snake. Other well known ACE inhibitors such as captopril and analopril were developed based on modifications to the venom chemical structures.

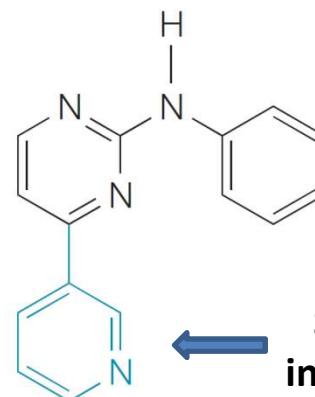
Rational drug design approach



Example: The drug *Gleevec* for the treatment of CML was found by screening a library of compounds against Protein Kinase C active. Compounds that showed activity against PKC were chemically modified based on computational modelling studies and X-ray structures of their interaction with the target for CML, the BCR-ABL kinase. The molecules were modified until a compound with sufficient inhibitory potency against BCR-ABL, safety and bioavailability was found.

Rational design of Glivec (Imatinib)

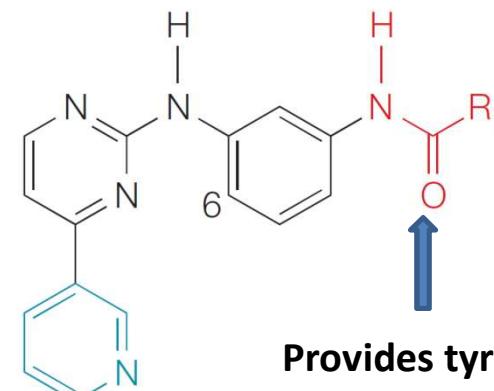
Found on a screen against PKC activity:



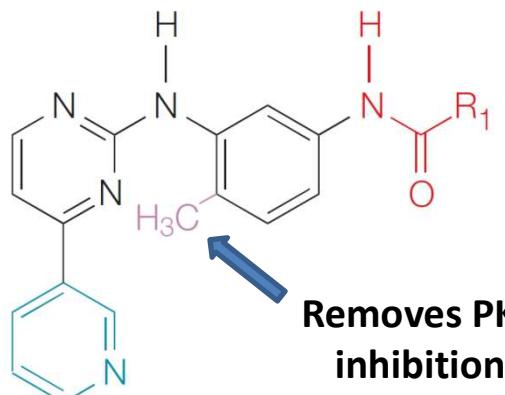
Strong inhibition against PKC



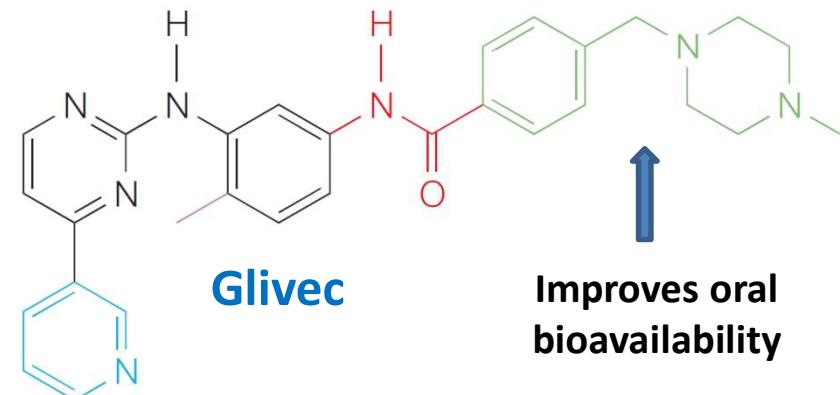
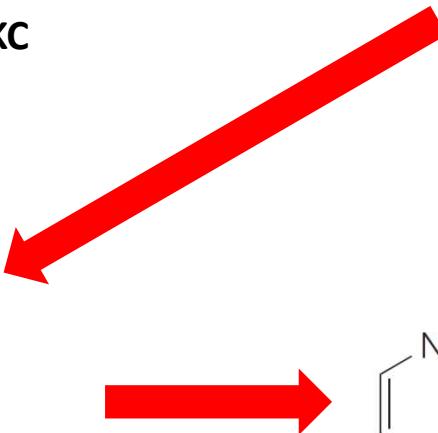
Chronic Mielogenous Leukemia



Provides tyrosine kinase inhibition



Removes PKC inhibition



Glivec

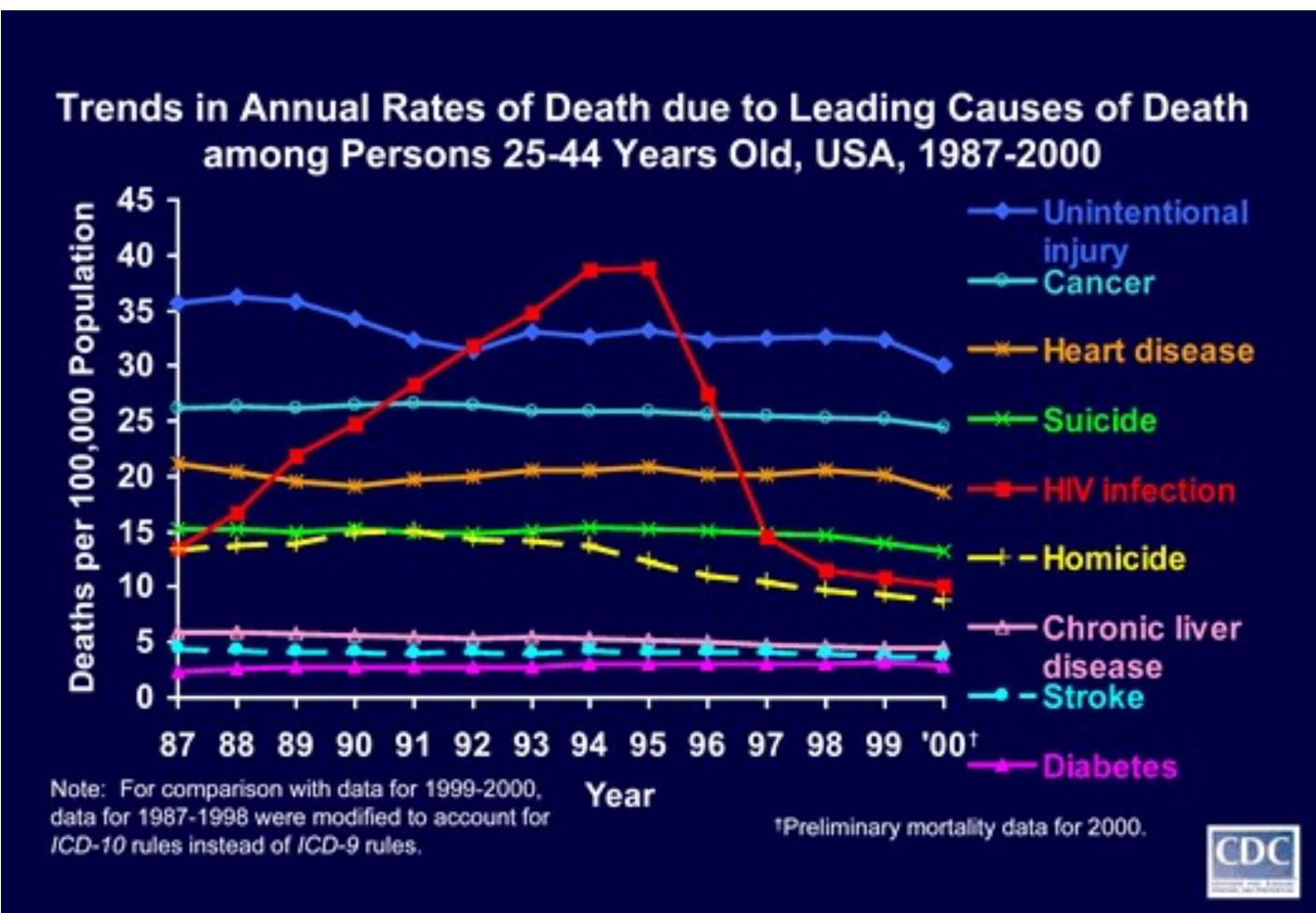
Improves oral bioavailability

BCR-ABL kinase inhibition

Structure *versus* Ligand-Based Drug Design

	Known Ligands	Unknown Ligands
Know protein structure	<p>Structure-based drug design (SBDD)</p> <p>Protein modelling Docking-guided Chemical optimization</p>	<p><i>De novo</i> design</p>
Unknown protein structure	<p>Ligand-based drug design (LBDD)</p> <p>1 or more ligands</p> <ul style="list-style-type: none">• Similarity searching <p>Several ligands</p> <ul style="list-style-type: none">• Pharmacophore searching <p>Many ligands (20+)</p> <ul style="list-style-type: none">• Quantitative Structure-Activity Relationships (QSAR)	<p>No rational approach</p> <p>Need experimental data of some sort</p> <p>Can apply ADMET filters</p>

ADMET: absorption, distribution, metabolism, excretion, toxicity



The trend in HIV infection reflects the advances in computer-aided drug design

Q: “Is there really a case where a drug that’s on the market was designed by a computer ?”

A: “No”

Jorgensen, WL (2004) “The many roles of computation in drug discovery” *Science* 303:813

Computational modelling techniques cannot *per se* finding new drugs (not yet, at least), but they are na immensely and indispensable tool in the process of drug discovery!

A Simulação Computacional pode guiar o processo de síntese e optimização de compostos...

Journal of
**Medicinal
Chemistry**

Article

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Computationally-Guided Optimization of a Docking Hit to Yield Catechol Diethers as Potent Anti-HIV Agents

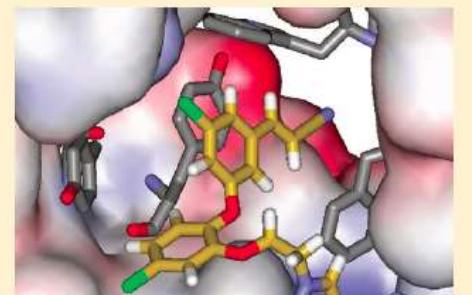
Mariela Bollini,[†] Robert A. Domaoal,[‡] Vinay V. Thakur,[†] Ricardo Gallardo-Macias,[†] Krasimir A. Spasov,[‡] Karen S. Anderson,^{*,‡} and William L. Jorgensen^{*,†}

[†]Department of Chemistry, Yale University, New Haven, Connecticut 06520-8107, United States

[‡]Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut 06520-8066, United States

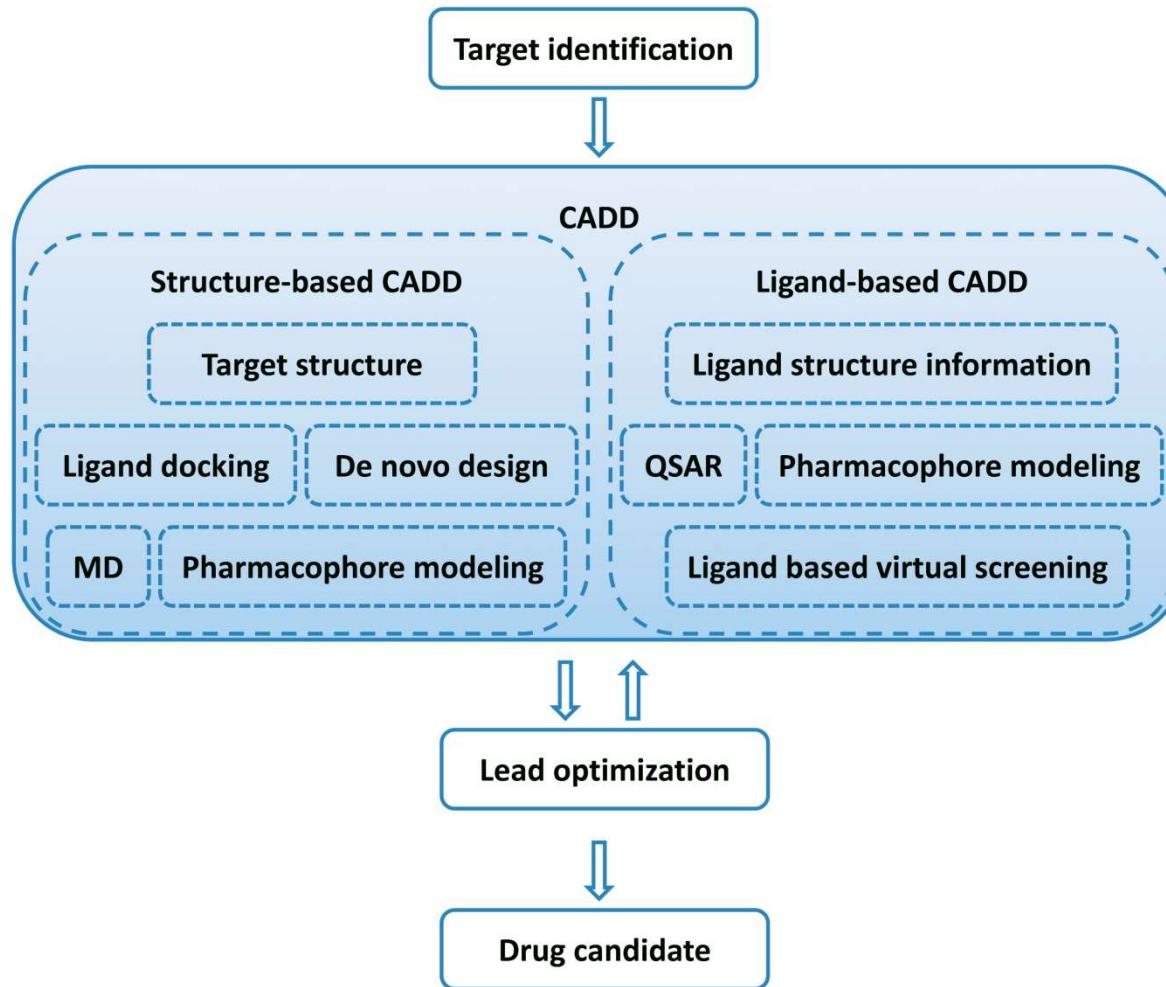
 Supporting Information

ABSTRACT: A 5- μ M docking hit has been optimized to an extraordinarily potent (55 pM) non-nucleoside inhibitor of HIV reverse transcriptase. Use of free energy perturbation (FEP) calculations to predict relative free energies of binding aided the optimizations by identifying optimal substitution patterns for phenyl rings and a linker. The most potent resultant catechol diethers feature terminal uracil and cyanovinylphenyl groups. A halogen bond with Pro95 likely contributes to the extreme potency of compound 42. In addition, several examples are provided illustrating failures of attempted grafting of a substructure from a very active compound onto a seemingly related scaffold to improve its activity.

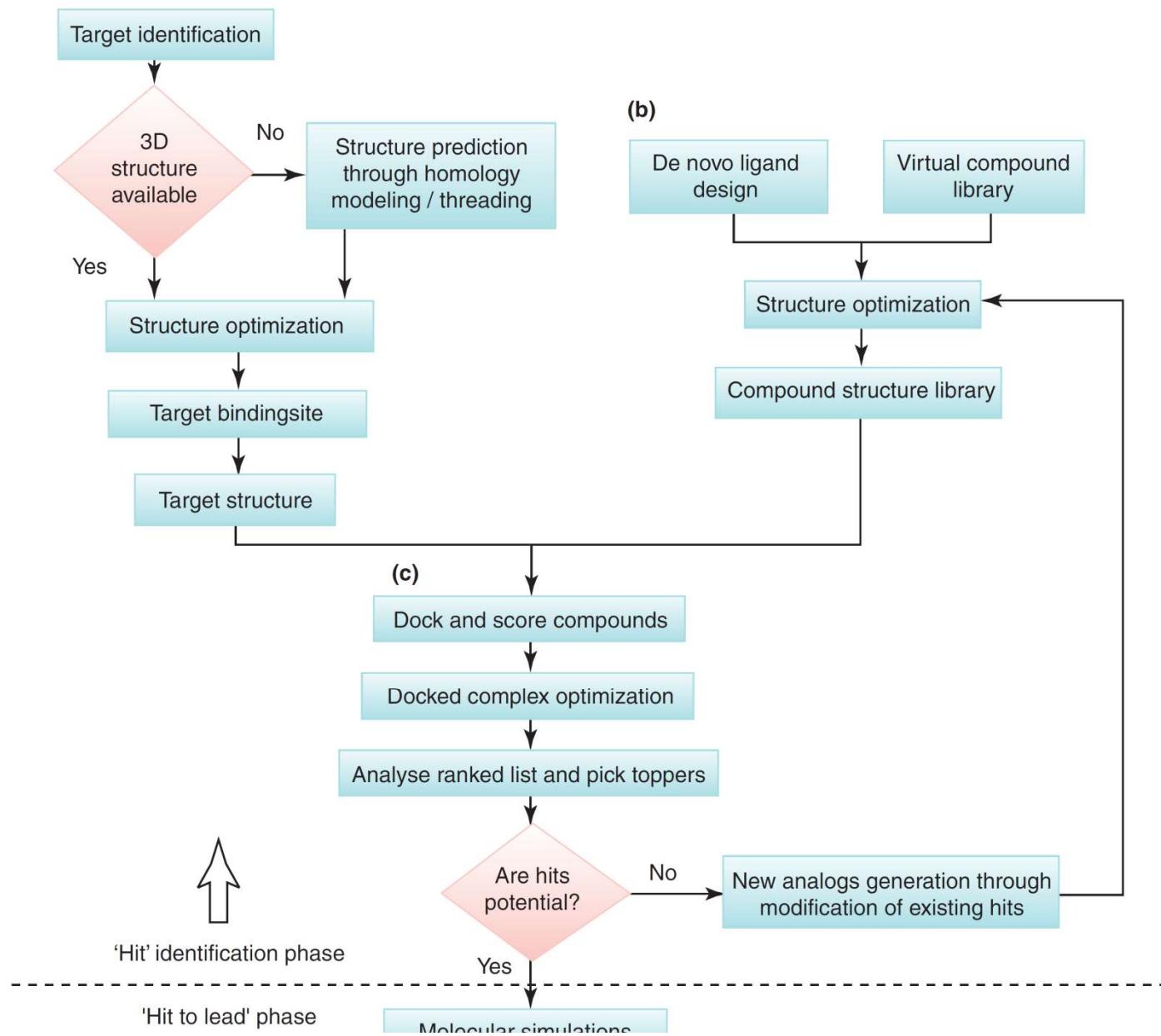


Bollini M (2011) *J Med Chem* 54:8582

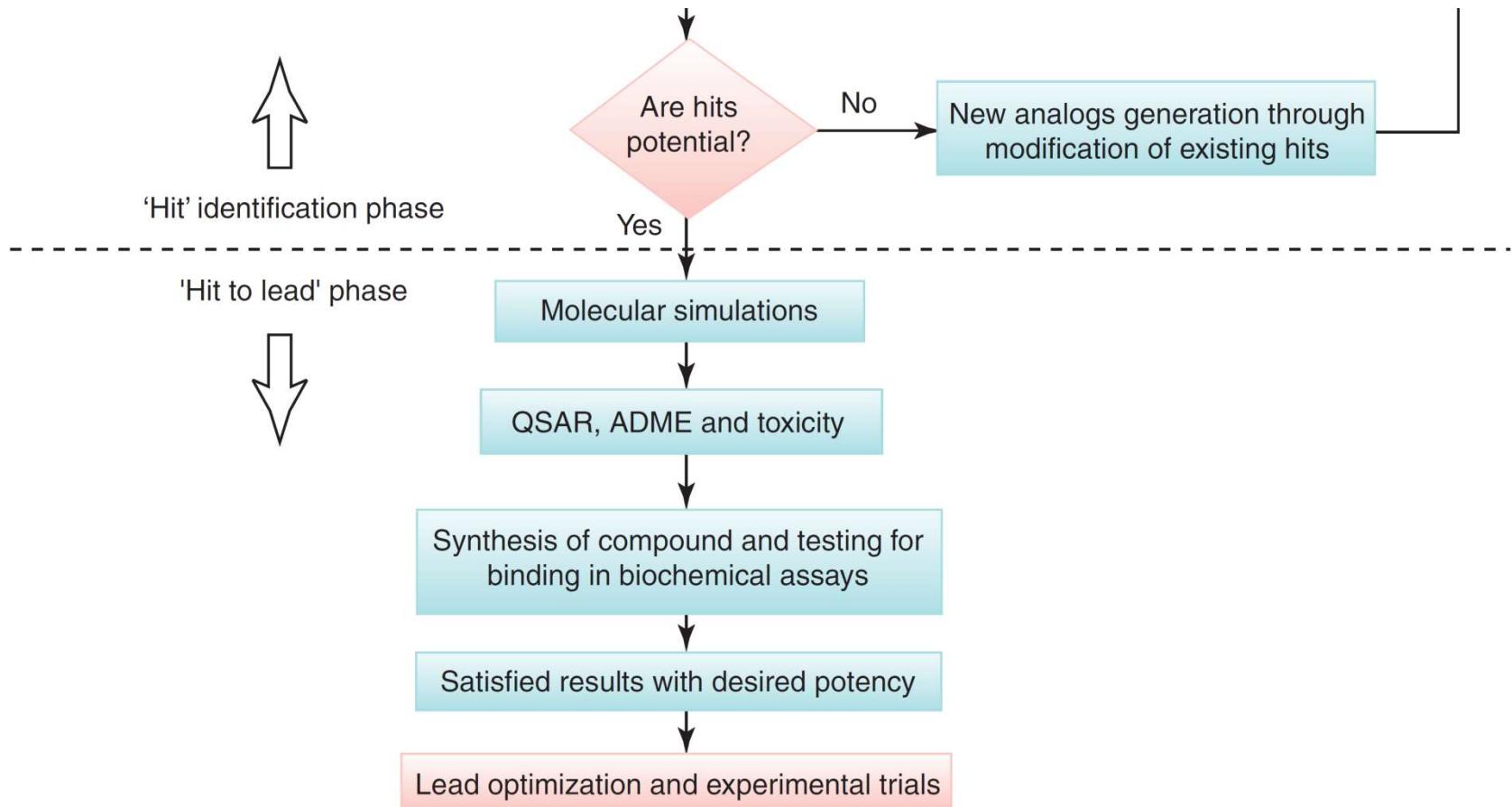
CADD in drug discovery/design pipeline



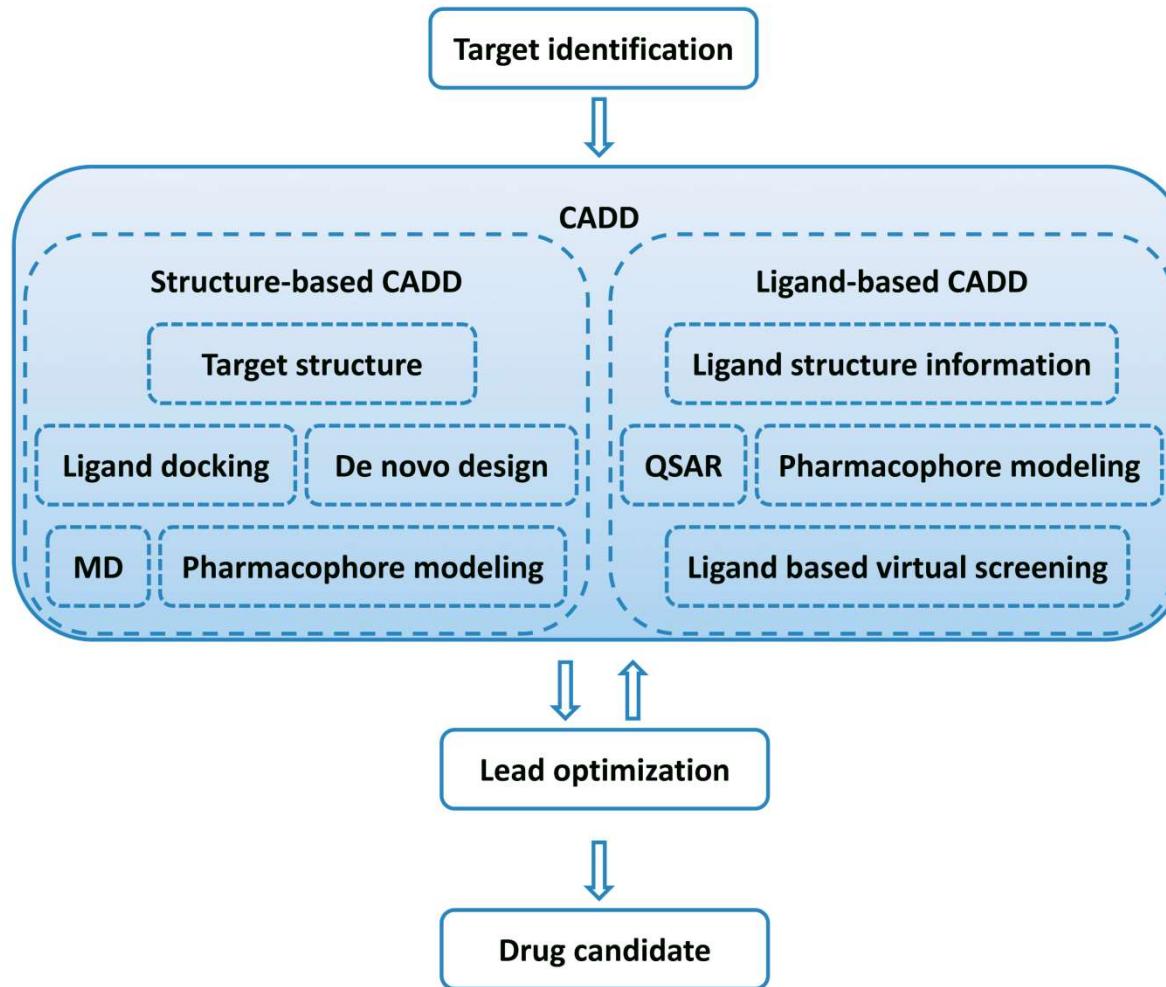
SBDD – Hit Identification phase



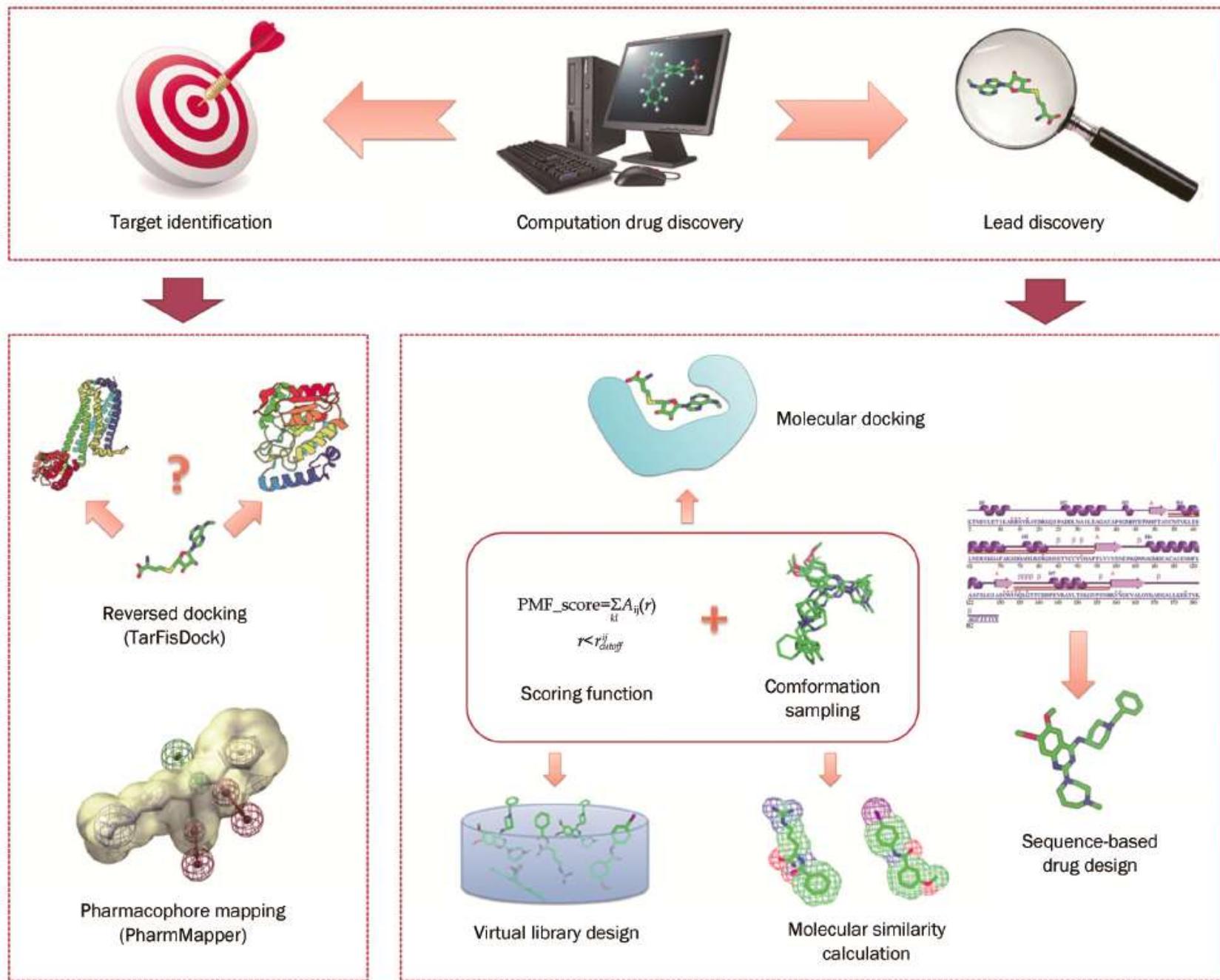
SBDD – Hit to lead phase

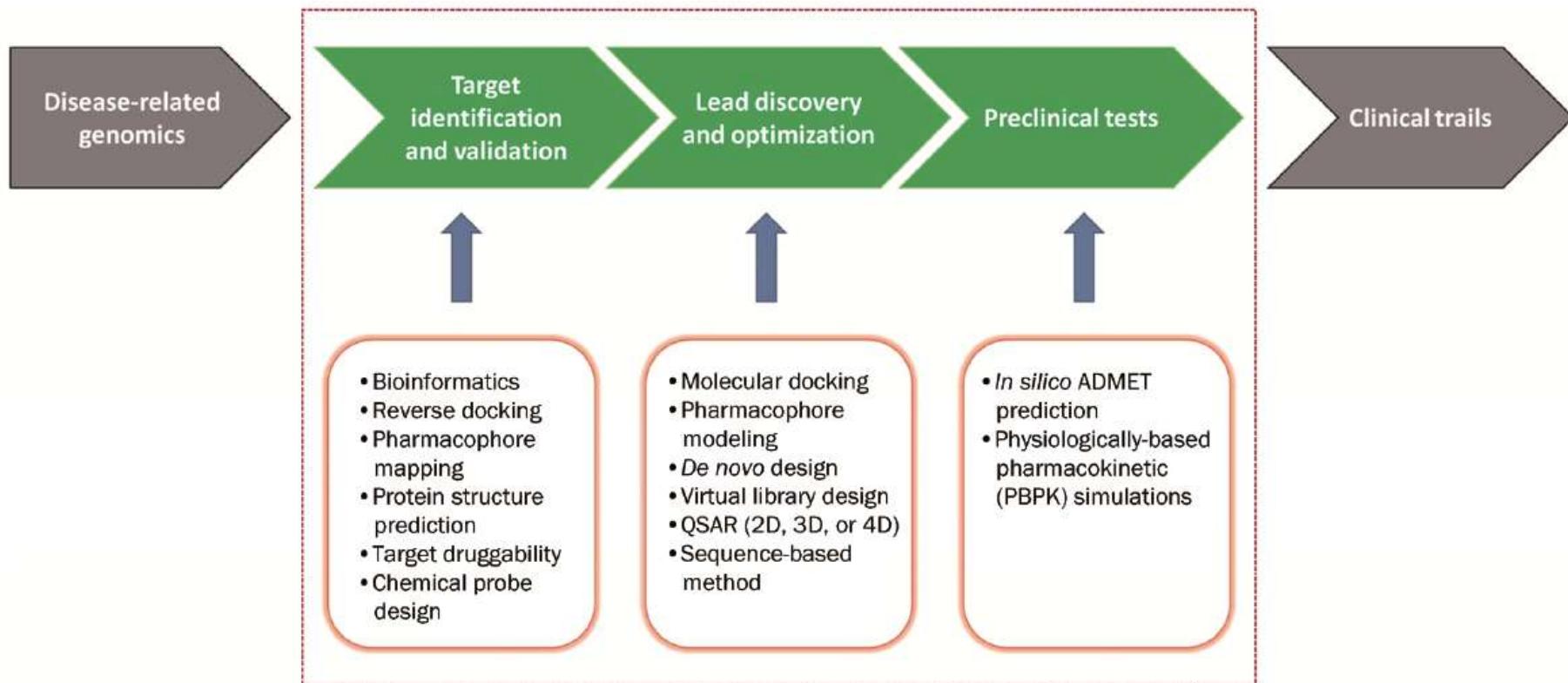


CADD in drug discovery/design pipeline



CADD – Computer-Aided Drug Design





Chemical compound repositories for virtual screening

Database	Type	Size
PubChem (Wheeler et al., 2006)	Biologic activities of small molecules	~40,000,000
Accelrys Available Chemicals Directory (ACD) (Accelrys, 2012)	Consolidated catalog from major chemical suppliers	~7,000,000
PDBeChem (Dimitropoulos, 2006)	Ligands and small molecules referred in PDB	14,572
Zinc (Irwin and Shoichet, 2005)	Annotated commercially available compounds	~21,000,000
LIGAND (Goto et al., 2002)	Chemical compounds with target and reactions data	16,838
DrugBank (Wishart et al., 2006)	Detailed drug data with comprehensive drug target information	6711
ChemDB (Chen et al., 2005, 2007)	Annotated commercially available molecules	~5,000,000
WOMBAT Data base (World of Molecular BioAcTivity) (Ekins et al., 2007; Hristozov et al., 2007)	Bioactivity data for compounds reported in medicinal chemistry journals	331,872
MDDR (MDL Drug Data Report) (Hristozov et al., 2007)	Drugs under development or released; descriptions of therapeutic	180,000
3D MIND (Mandal et al., 2009).	Molecules with target interaction and tumor cell line screen data	100,000

Chemical databases

TABLE 2

Chemical structure/small molecule database

Database	Number of records	Sub-structure search	Structure formats	Similarity search	Molecular descriptors	Clustering	Experimental data info
Pubchem	>37 Million(C) > 70 Million(S)	Yes	Smiles, SDF, 2D, 3D	Yes	Yes	Yes	Yes
Drug Bank	Approx.4800	Yes	Smiles, SDF, 2D, 3D, Mol, PDB	Yes	Yes	No	Yes
SPRESI ^{web}	7 million	Yes	SDF, PDF	Yes	Yes	No	Yes
KEGG Ligand	17,627	Yes	Mol, KCF	Yes	Yes*	No	Yes
Chem DB	>600,000	Yes	Mol, SDF	Yes	Yes	No	No
Protein ligand database v1.3	485	No	PDB	No	No	No	Yes
Chem Bank	2344	Yes	Smiles, Smarts	Yes	Yes	No	Yes
ChemPDB	8702	Yes	Smiles	Yes	No	No	Yes
ChemSpider	>20 Million	Yes	Mol, Smiles, SDF, SKC, CDX	Yes	Yes	No	Yes
ChemIDplus	>380,000	Yes	SDF, Mol, PDB, Smiles, RDF	Yes	Yes	No	Yes*
Zinc	>2.7 Million	Yes	Smiles, Mol2, SDF	No	Yes*	No	Yes*
Timetec's bioscreening database	600,000	Yes	Mol, SDF	Yes	No	No	No
PDBbind	3214	Yes	Smiles	Yes	BD	No	Yes
AffinDB	748	No	PDB	No	BD	No	Yes
BindingMoad	~9000	No	PDB, SDF, Smiles, Mol	No	BD	No	Yes
BindingDB	~11,000	Yes	SDF, PDB, Mol, Smiles	Yes	Yes	No	Yes

Yes*: information from other linked database. Abbreviation: BD, binding data.

Software para virtual screening

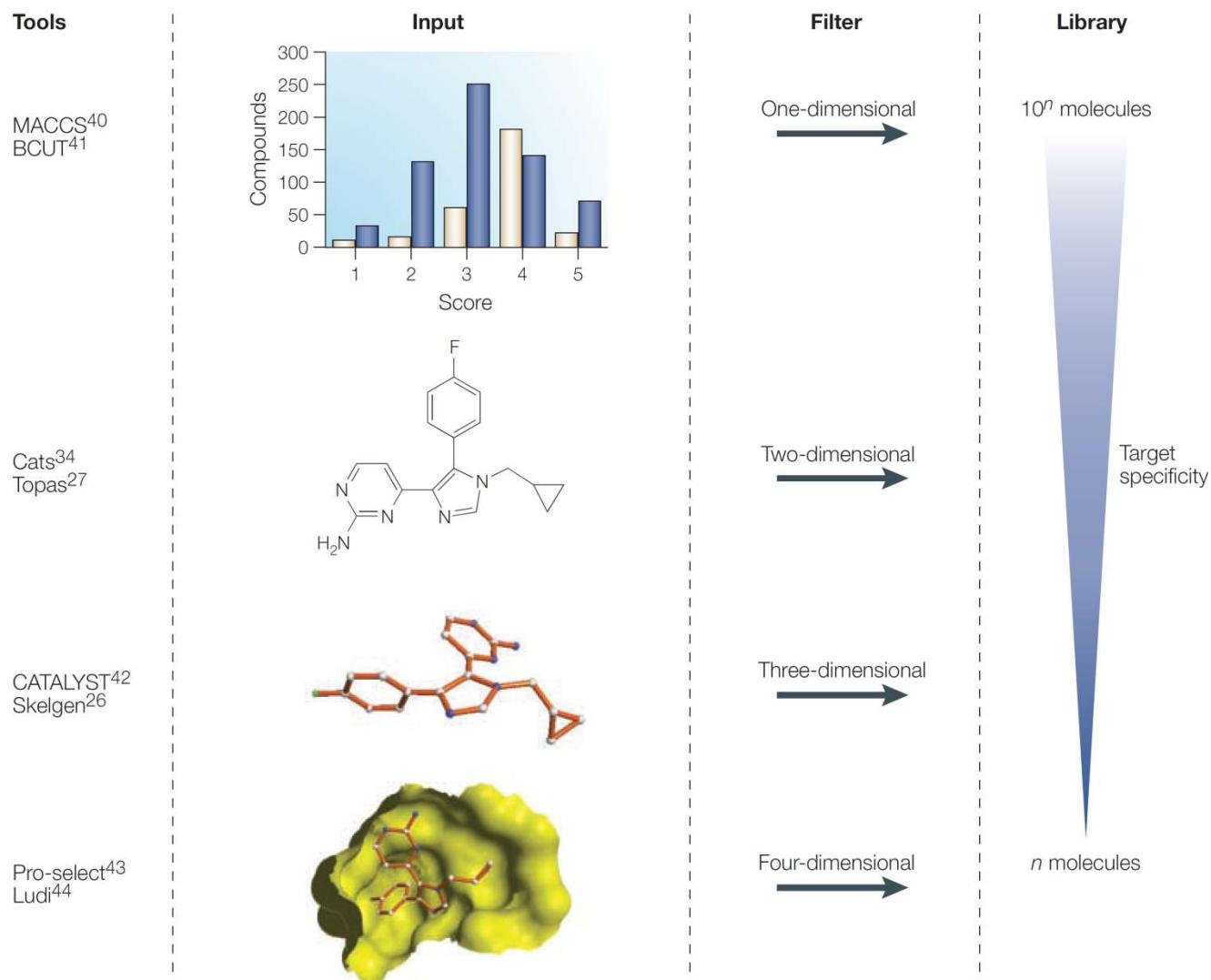
TABLE 3

Molecular docking tools for virtual screening

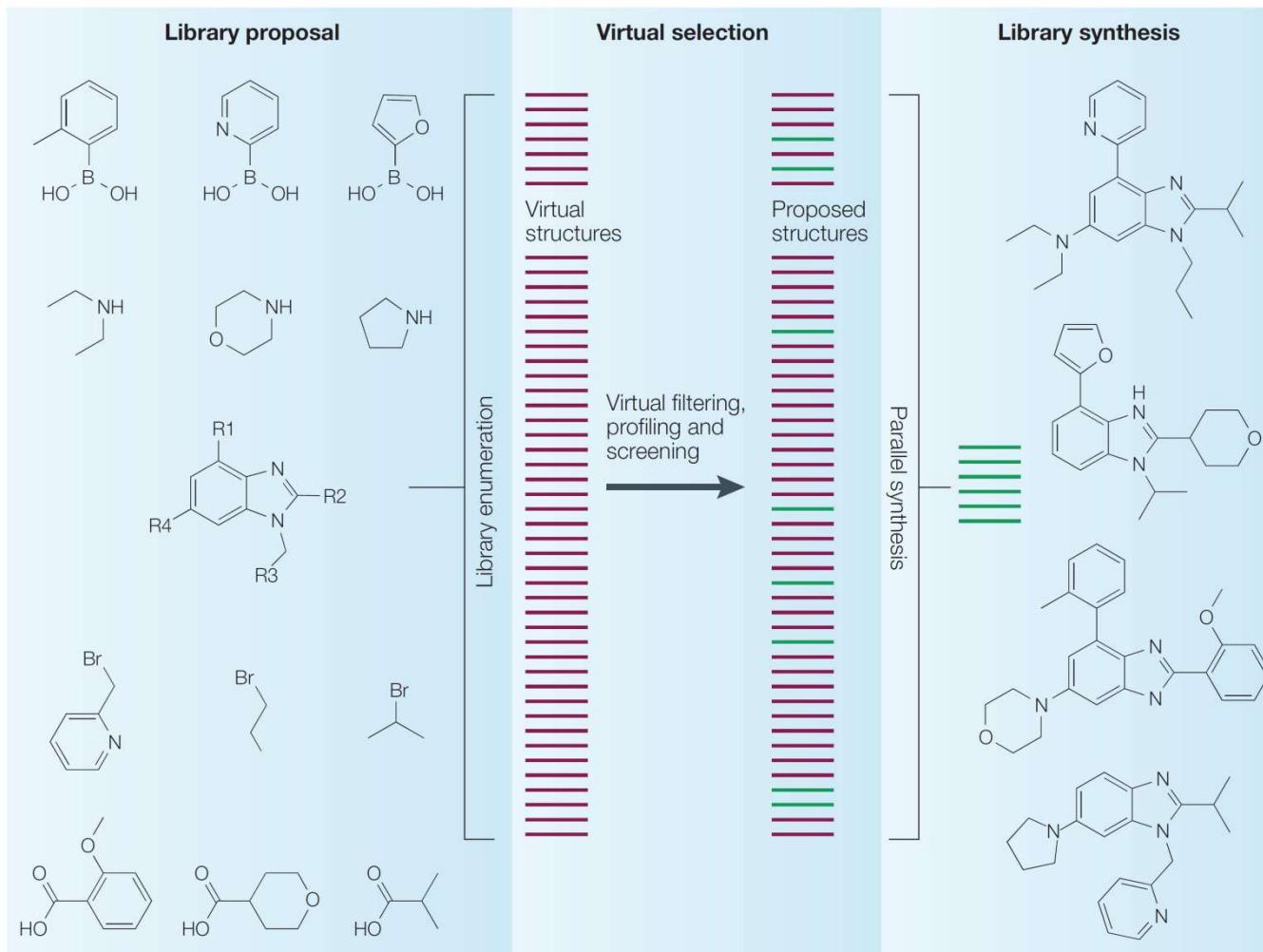
Software	Method	Source	Application
AutoDock	Lamarckian genetic algorithm	http://autodock.scripps.edu/	PL
Gold	Genetic algorithm	http://www.ccdc.cam.ac.uk/	PL
FRED	Directed docking with SMARTS ^a patterns	http://www.eyesopen.com/fred	PL
FlexX	SIS-algorithm ^b	http://www.biosolveit.de/FlexX/	PL
Dock Vision	Hybrid evolutionary algorithm	http://dockvision.com/	PL
Surflex-Dock	Hammerhead docking system	http://www.tripos.com/index.php?family=modules,SimplePage,,&page=surflex_dock&s=0	PL
Situs	Correlation-based rigid body docking and density filtering	http://situs.biomachina.org/index.html	ARD
DOCK	Geometric matching algorithm	http://dock.compbio.ucsf.edu/	PPL
GRAMM	Empirical approach	http://vakser.bioinformatics.ku.edu/main/resources_gramm.php	PPL
ICM-Docking	Flexible docking	http://www.molsoft.com/docking.html	PPL
3D-Dock Suite	Fourier correlation algorithm, self consistent mean field optimization procedure, single distance constraint empirically derived pair potential	http://www.sbg.bio.ic.ac.uk/docking/index.html	PP
BiGGER	Soft-docking	http://www.cqfb.fct.unl.pt/	PP
DOT	Fast Fourier transforms	http://www.sdsc.edu/CCMS/DOT/	PP
ArgusLab	Genetic algorithm and ArgusDock	http://www.arguslab.com	PP
HADDOCK	Uses ambiguous interaction restraints of NMR	http://www.nmr.chem.uu.nl/haddock/	PP
Hex	Similar to conventional fast Fourier transform (FFT)	http://www.loria.fr/~ritchied/hex/	PP
rDock	Weighted sum of intermolecular, ligand intramolecular, site intramolecular and external restraint terms	http://www.ysbl.york.ac.uk/rDock	PL and RNA-ligand

Abbreviations: PL, protein–ligand docking software; PPL, protein–protein and protein–ligand docking software; PP, protein–protein docking software; ARD, atomic resolution structure docking software.

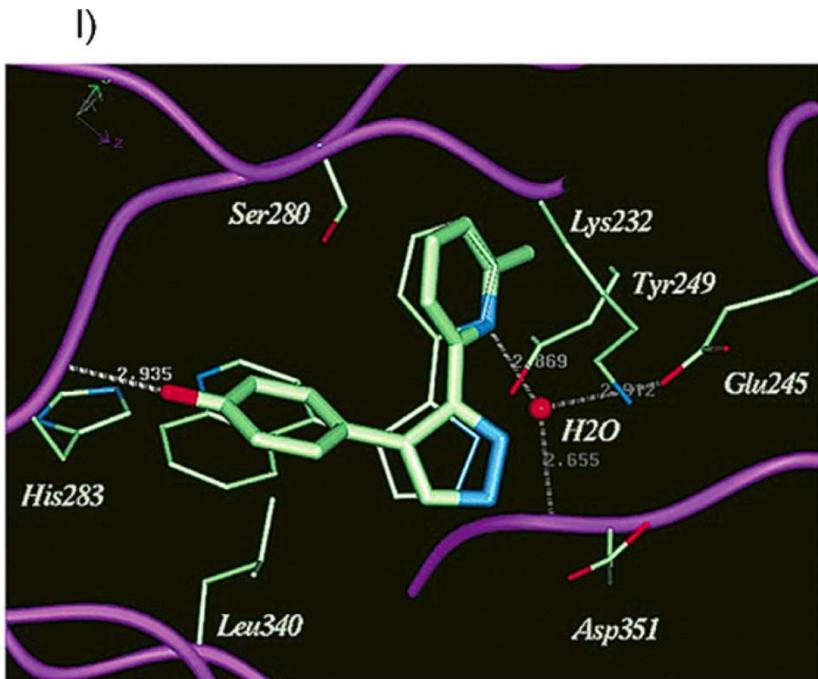
Filters in virtual screening



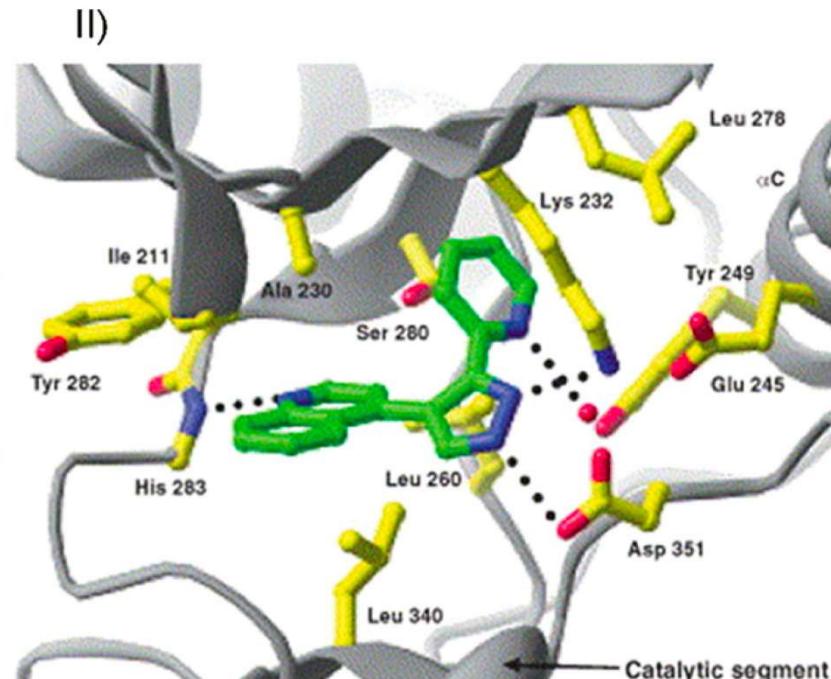
Filtering virtual compound libraries



Virtual screening can produce the same results as real screening



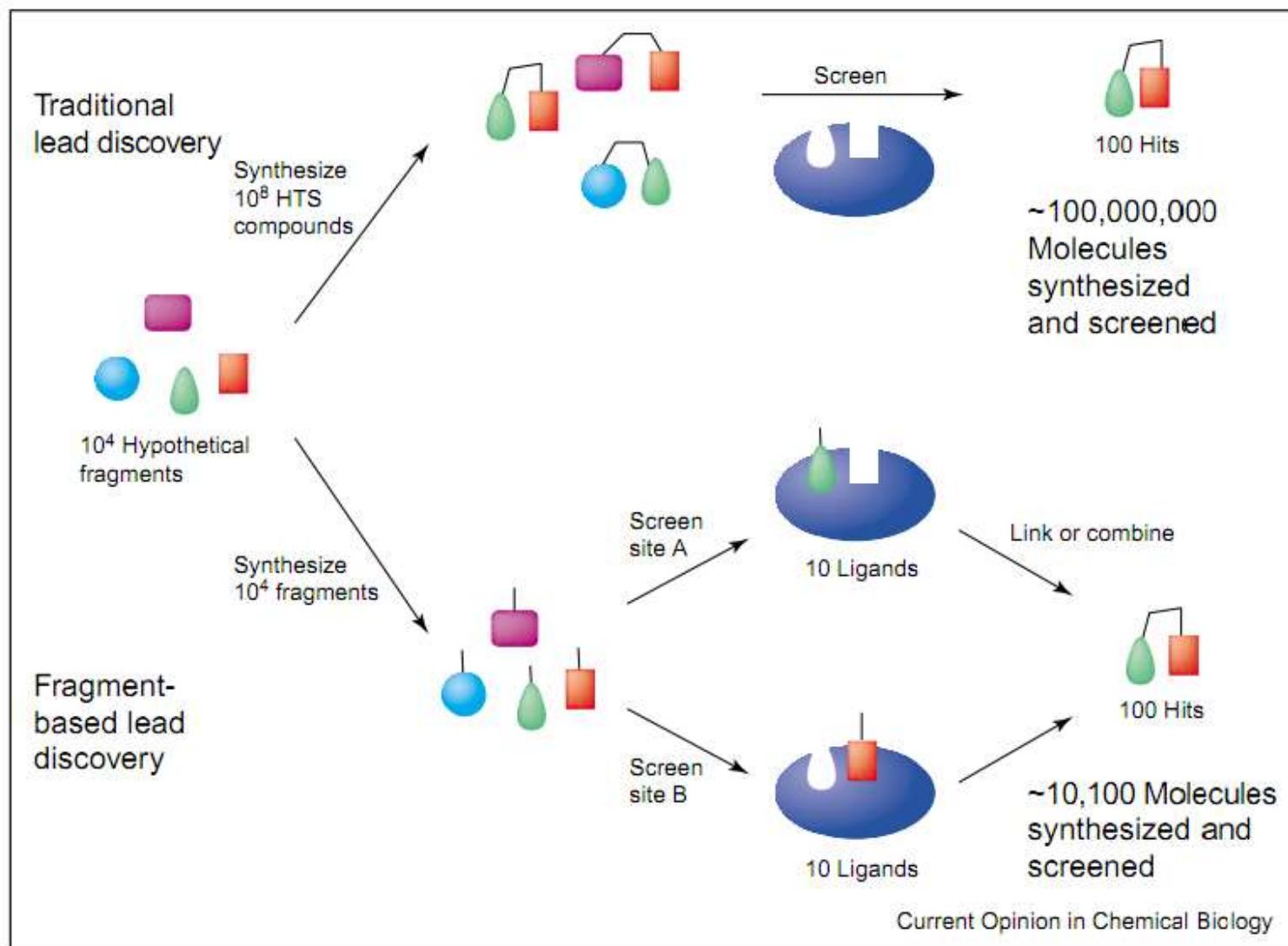
High-throughput screening



Virtual screening

TbR-I kinase domain

Fragment search vs traditional search



Computationally-Guided Optimization of a Docking Hit to Yield Catechol Diethers as Potent Anti-HIV Agents

Mariela Bollini,[†] Robert A. Domaao,[‡] Vinay V. Thakur,[†] Ricardo Gallardo-Macias,[†] Krasimir A. Spasov,[‡] Karen S. Anderson,^{*,‡} and William L. Jorgensen^{*,†}

[†]Department of Chemistry, Yale University, New Haven, Connecticut 06520-8107, United States

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 *Supporting Information*

ABSTRACT: A 5- μ M docking hit has been optimized to an extraordinarily potent (55 pM) non-nucleoside inhibitor of HIV reverse transcriptase. Use of free energy perturbation (FEP) calculations to predict relative free energies of binding aided the optimizations by identifying optimal substitution patterns for phenyl rings and a linker. The most potent resultant catechol diethers feature terminal uracil and cyanovinylphenyl groups. A halogen bond with Pro95 likely contributes to the extreme potency of compound 42. In addition, several examples are provided illustrating failures of attempted grafting of a substructure from a very active compound onto a seemingly related scaffold to improve its activity.

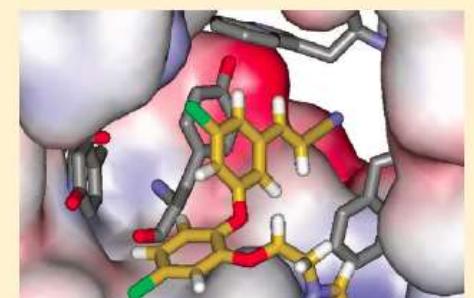


TABLE 1**Drug discovery software packages**

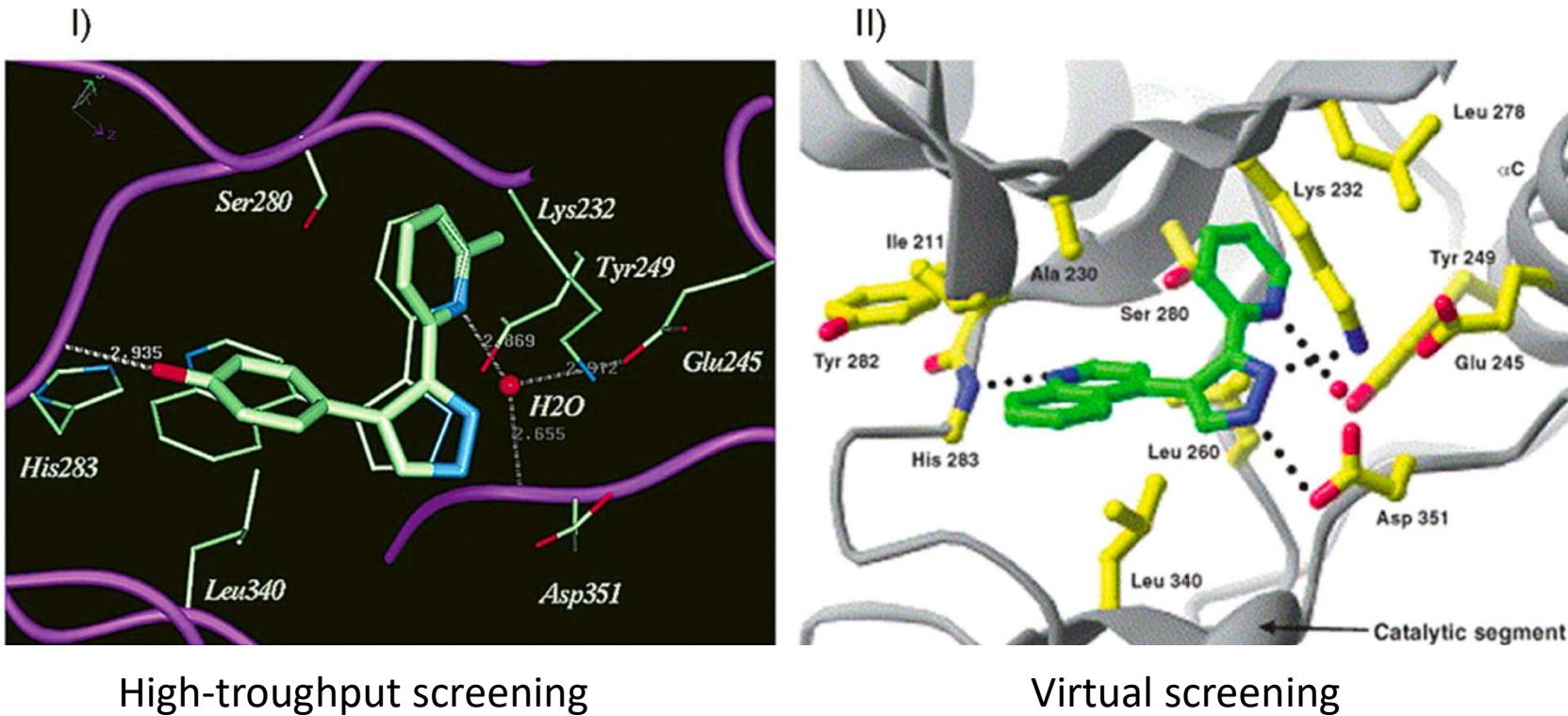
Software	Features											Source
	TS	BS	MB	DB	MM	Dc	Pc	MD	QSAR	Sc	ADME	
Discovery studio	×	×	×		×	×	×	×	×	×	×	http://accelrys.com/
Biograf		×	×		×	×	×	×	×		×	http://www.biograf.ch/
BiosolveIT		×	×			×	×			×		http://www.biosolveit.de/
Sybyl	×		×			×	×	×	×	×	×	http://www.optive.com/
Forecaster 2.6.1	×					×	×			×		http://www.fitted.ca/
Aurora drug discovery software		×			×	×		×				http://www.aurorafinechemicals.com/
Spartan			×	×	×		×			×	×	http://www.computational-chemistry.co.uk/
ChemTree			×				×		×	×	×	http://www.goldenhelix.com/
Quantum	×					×		× ¹		×	×	http://www.q-pharm.com/
Molecular modeling platform	×	×	×	×	×	×	×	×	×	×		https://www.schrodinger.com/
MoE	×	×	×	×	×	×	×	×	×	×		http://www.chemcomp.com/
Hyperchem	×		×		×	×		×		×		http://www.hyper.com/

Abbreviations: TS, target 3D structure prediction; BS, binding site prediction; MB, molecule builder; DB, database search; MM, molecular mechanics; Pc, pharmacophore; MD, molecular dynamics simulation; QSAR, quantitative structure activity relationship; Sc, screening; ADME, absorption distribution metabolism excretion; TP, toxicity prediction; [x¹: selective channels].

Estratégias computacionais de identificação de *hits*

- Docking
- Virtual screening
- *De novo* design

Virtual screening can produce the same results as real screening



TbR-I kinase domain

Chemical databases

TABLE 2

Chemical structure/small molecule database

Database	Number of records	Sub-structure search	Structure formats	Similarity search	Molecular descriptors	Clustering	Experimental data info
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Yes*: information from other linked database. Abbreviation: BD, binding data.

Software para virtual screening

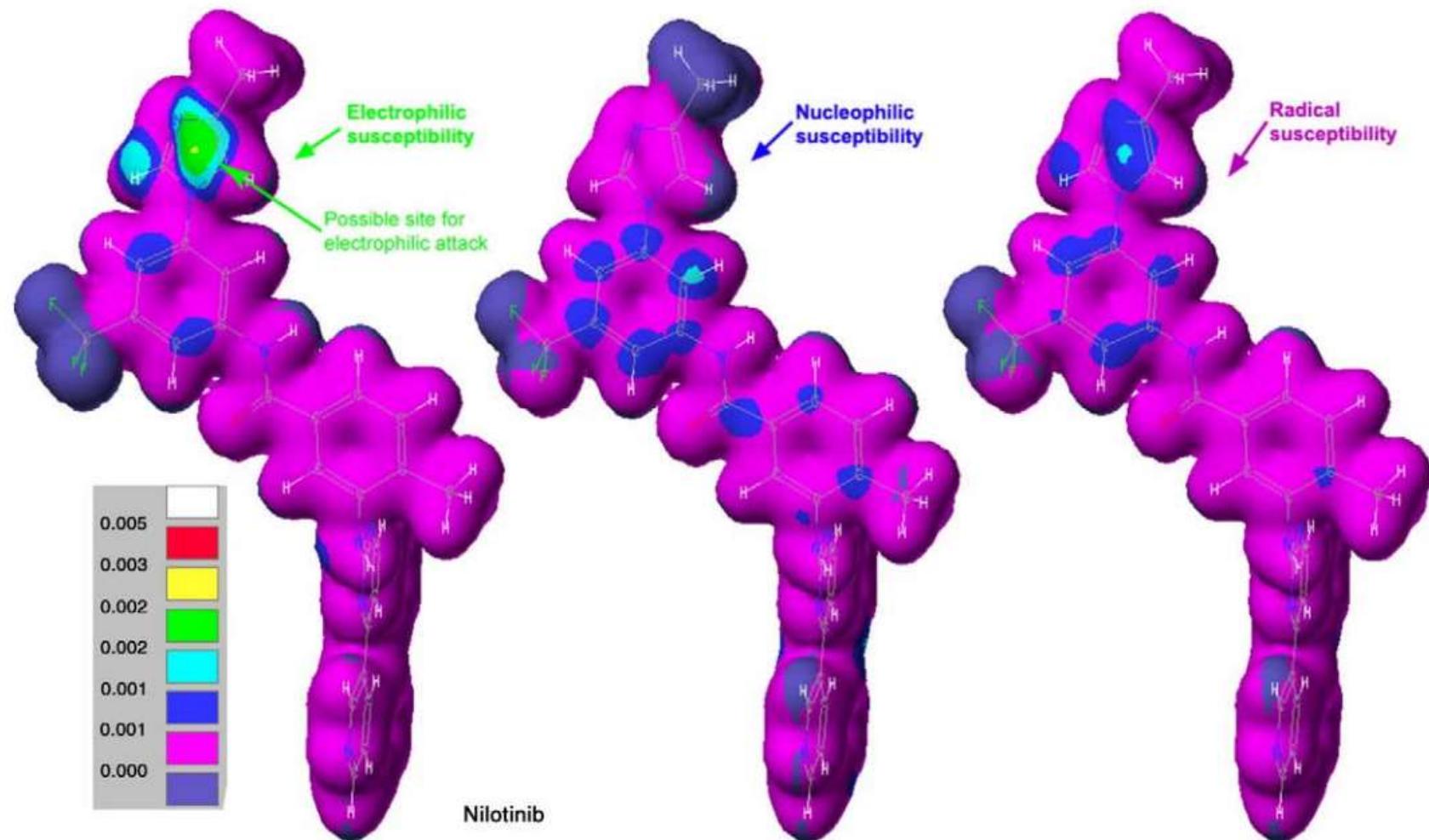
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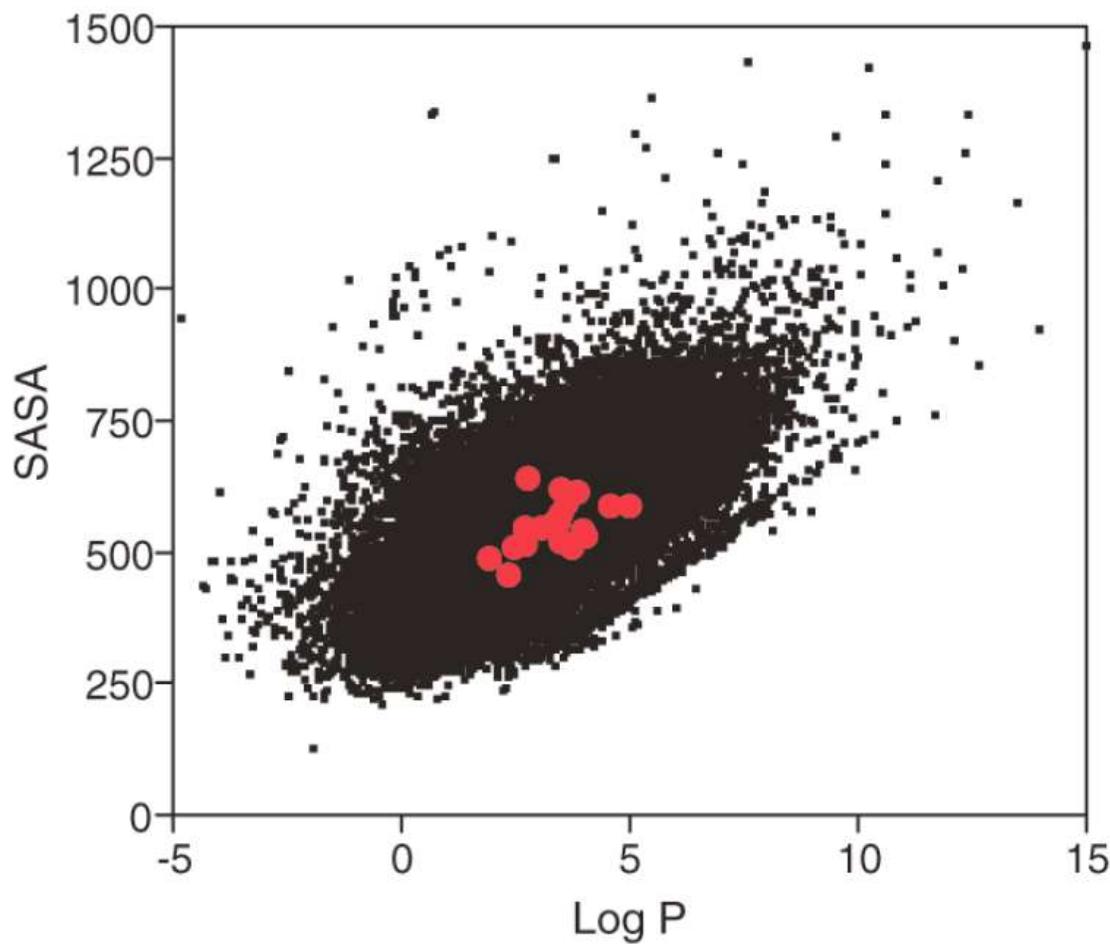
Software	Method	Source	Application
AutoDock	Lamarckian genetic algorithm	http://autodock.scripps.edu/	PL
Gold	Genetic algorithm	http://www.ccdc.cam.ac.uk/	PL
FRED	Directed docking with SMARTS ^a patterns	http://www.eyesopen.com/fred	PL
FlexX	SIS-algorithm ^b	http://www.biosolveit.de/FlexX/	PL
Dock Vision	Hybrid evolutionary algorithm	http://dockvision.com/	PL
Surflex-Dock	Hammerhead docking system	http://www.tripos.com/index.php?family=modules,SimplePage,,&page=surflex_dock&s=0	PL
Situs	Correlation-based rigid body docking and density filtering	http://situs.biomachina.org/index.html	ARD
DOCK	Geometric matching algorithm	http://dock.compbio.ucsf.edu/	PPL
GRAMM	Empirical approach	http://vakser.bioinformatics.ku.edu/main/resources_gramm.php	PPL
ICM-Docking	Flexible docking	http://www.molsoft.com/docking.html	PPL
3D-Dock Suite	Fourier correlation algorithm, self consistent mean field optimization procedure, single distance constraint empirically derived pair potential	http://www.sbg.bio.ic.ac.uk/docking/index.html	PP
BiGGER	Soft-docking	http://www.cqfb.fct.unl.pt/	PP
DOT	Fast Fourier transforms	http://www.sdsc.edu/CCMS/DOT/	PP
ArgusLab	Genetic algorithm and ArgusDock	http://www.arguslab.com	PP
HADDOCK	Uses ambiguous interaction restraints of NMR	http://www.nmr.chem.uu.nl/haddock/	PP
Hex	Similar to conventional fast Fourier transform (FFT)	http://www.loria.fr/~ritchied/hex/	PP
rDock	Weighted sum of intermolecular, ligand intramolecular, site intramolecular and external restraint terms	http://www.ysbl.york.ac.uk/rDock	PL and RNA-ligand

Abbreviations: PL, protein–ligand docking software; PPL, protein–protein and protein–ligand docking software; PP, protein–protein docking software; ARD, atomic resolution structure docking software.

Análise das propriedades moleculares



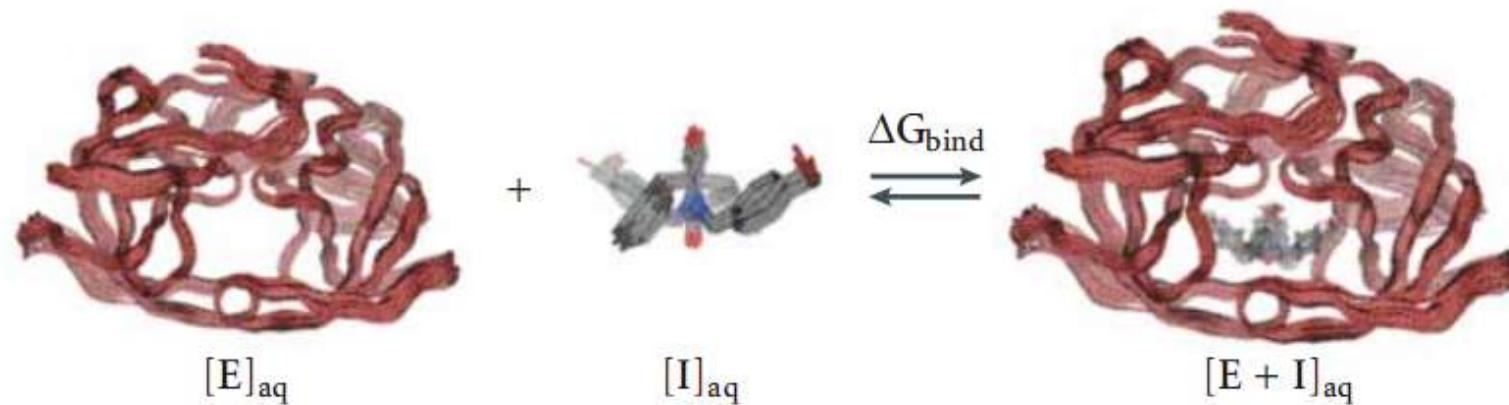
Filtragem do espaço de busca



Os pontos vermelhos representam vários inibidores da HIV-1 RT
(revelam semelhança na área e na polaridade)

Jorgensen, WL (2004) *Science* 303:813

A previsão da eficiência de ligação é essencial



$$\Delta G = -RT\ln K_A$$

$$K_A = K_i^{-1} = \frac{[EI]}{[E][I]}$$

Previsão teórica de constantes de dissociação

Journal of
**Medicinal
Chemistry**

Article

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Computationally-Guided Optimization of a Docking Hit to Yield Catechol Diethers as Potent Anti-HIV Agents

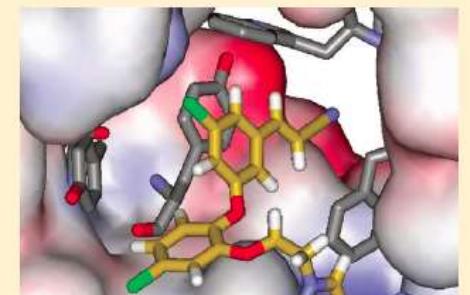
Mariela Bollini,[†] Robert A. Domaoal,[‡] Vinay V. Thakur,[†] Ricardo Gallardo-Macias,[†] Krasimir A. Spasov,[‡] Karen S. Anderson,^{*,‡} and William L. Jorgensen^{*,†}

[†]Department of Chemistry, Yale University, New Haven, Connecticut 06520-8107, United States

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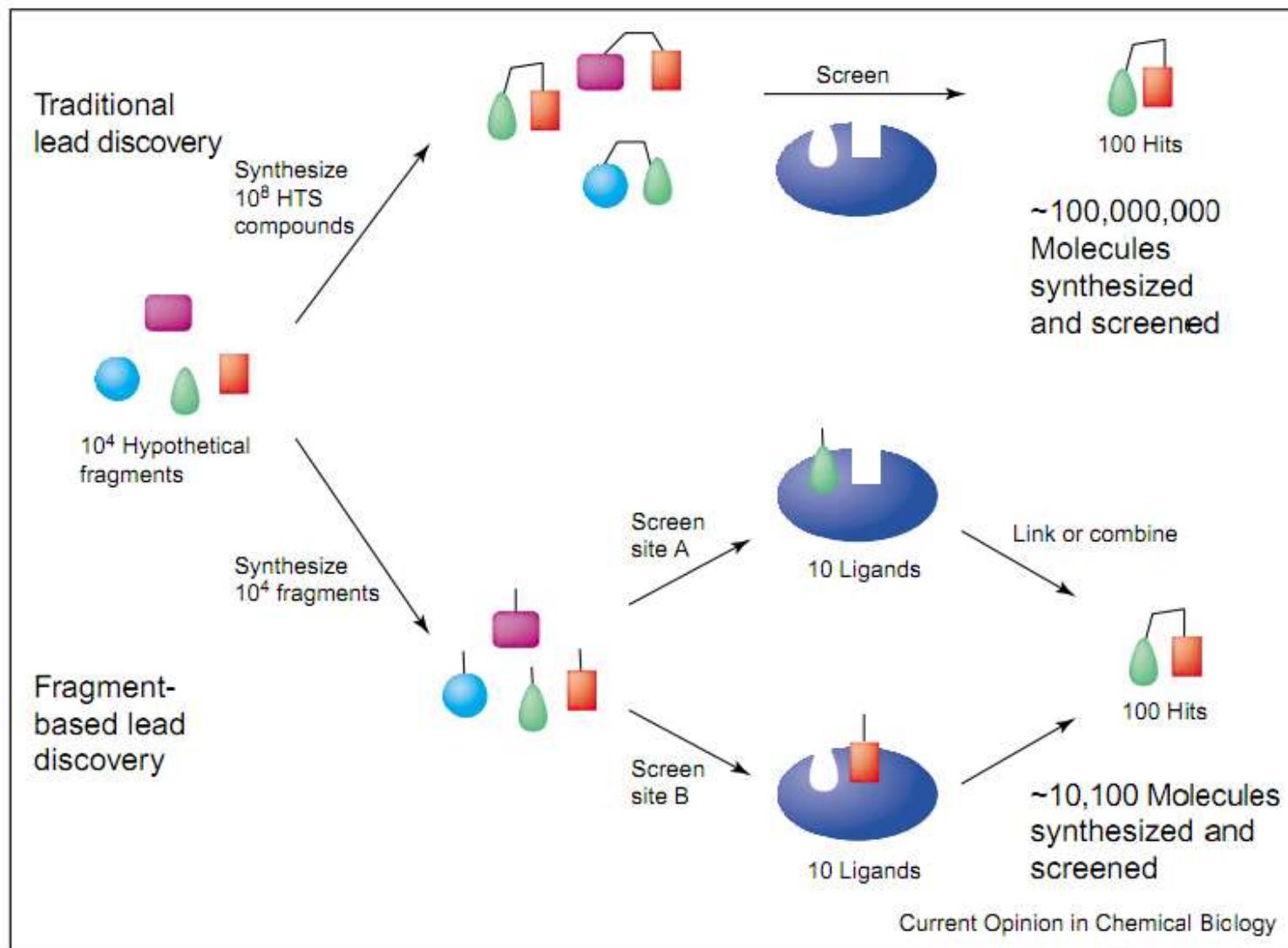
 Supporting Information

ABSTRACT: A 5- μ M docking hit has been optimized to an extraordinarily potent (55 pM) non-nucleoside inhibitor of HIV reverse transcriptase. Use of free energy perturbation (FEP) calculations to predict relative free energies of binding aided the optimizations by identifying optimal substitution patterns for phenyl rings and a linker. The most potent resultant catechol diethers feature terminal uracil and cyanovinylphenyl groups. A halogen bond with Pro95 likely contributes to the extreme potency of compound 42. In addition, several examples are provided illustrating failures of attempted grafting of a substructure from a very active compound onto a seemingly related scaffold to improve its activity.

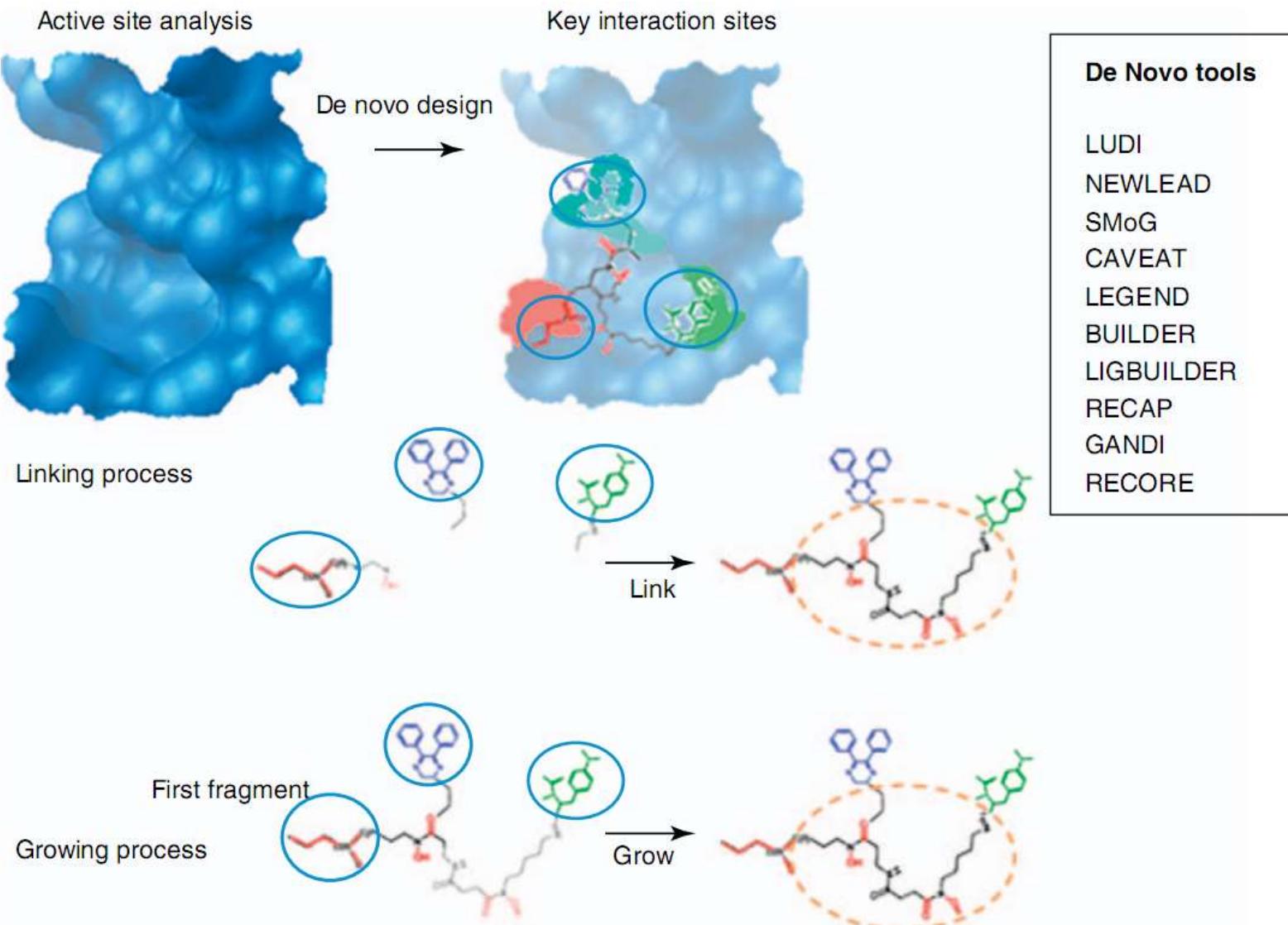


Bollini M (2011) *J Med Chem* 54:8582

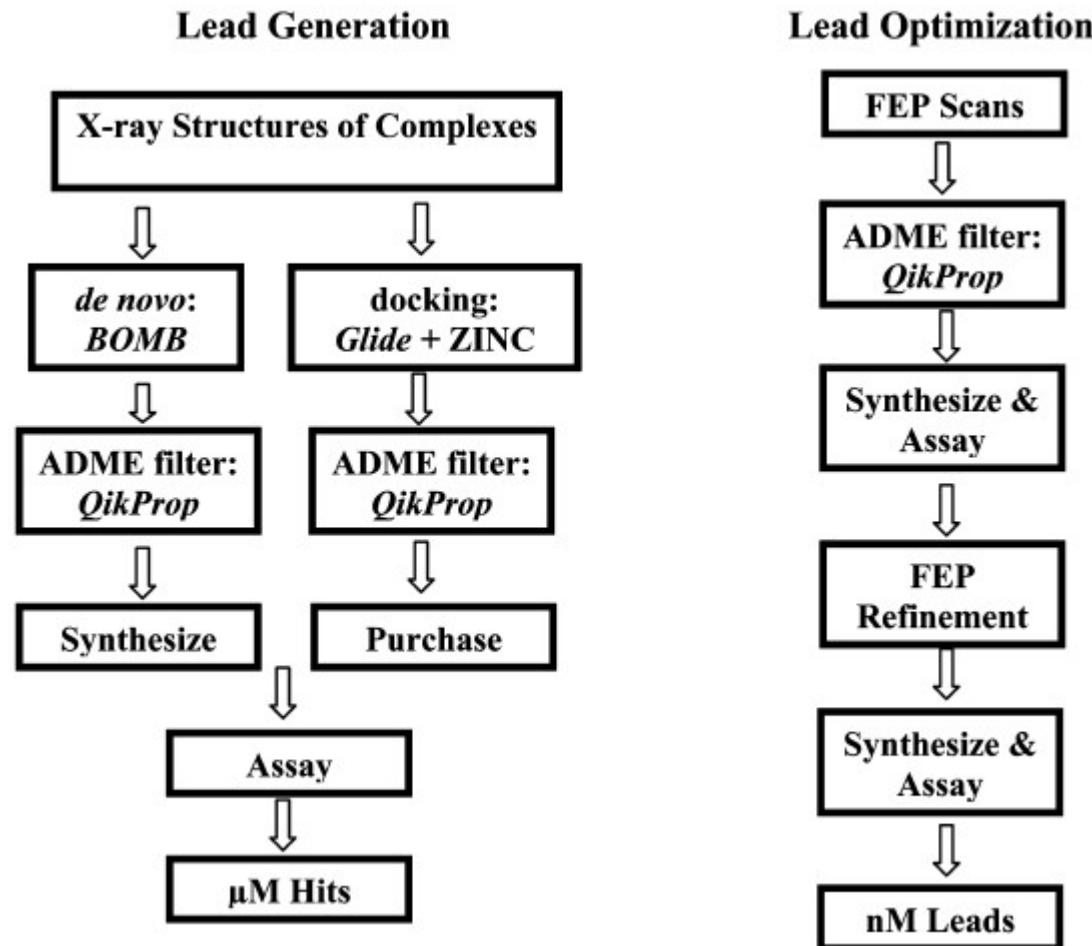
Pesquisa de fragmentos *versus* técnicas tradicionais



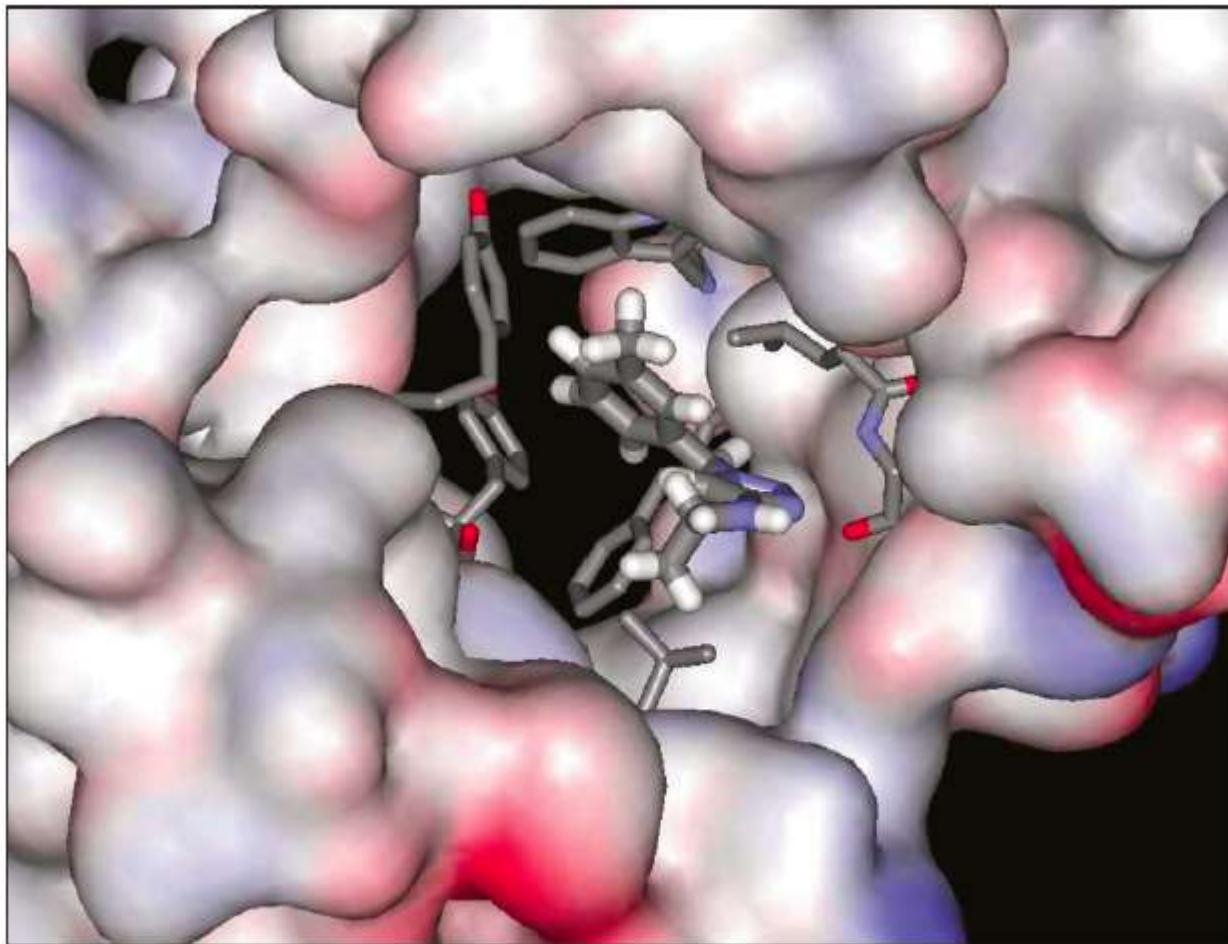
Desenho virtual de fármacos *de novo*



Molecular modeling in lead generation and optimization



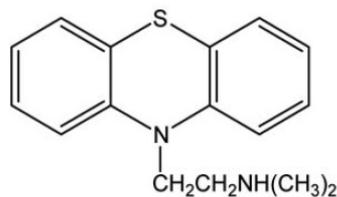
Jorgensen, WL (2004) *Acc Chem Res* 42:724



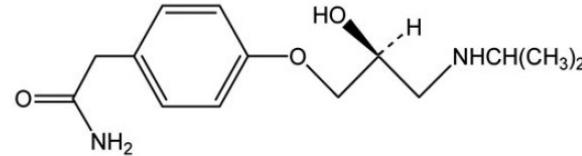
Inibidor construído no site HIV-1 RT a partir de uma molécula de amónia

Jorgensen, WL (2004) *Acc Chem Res* 42:724

Previsão de propriedades ADMET



chlorpromazine



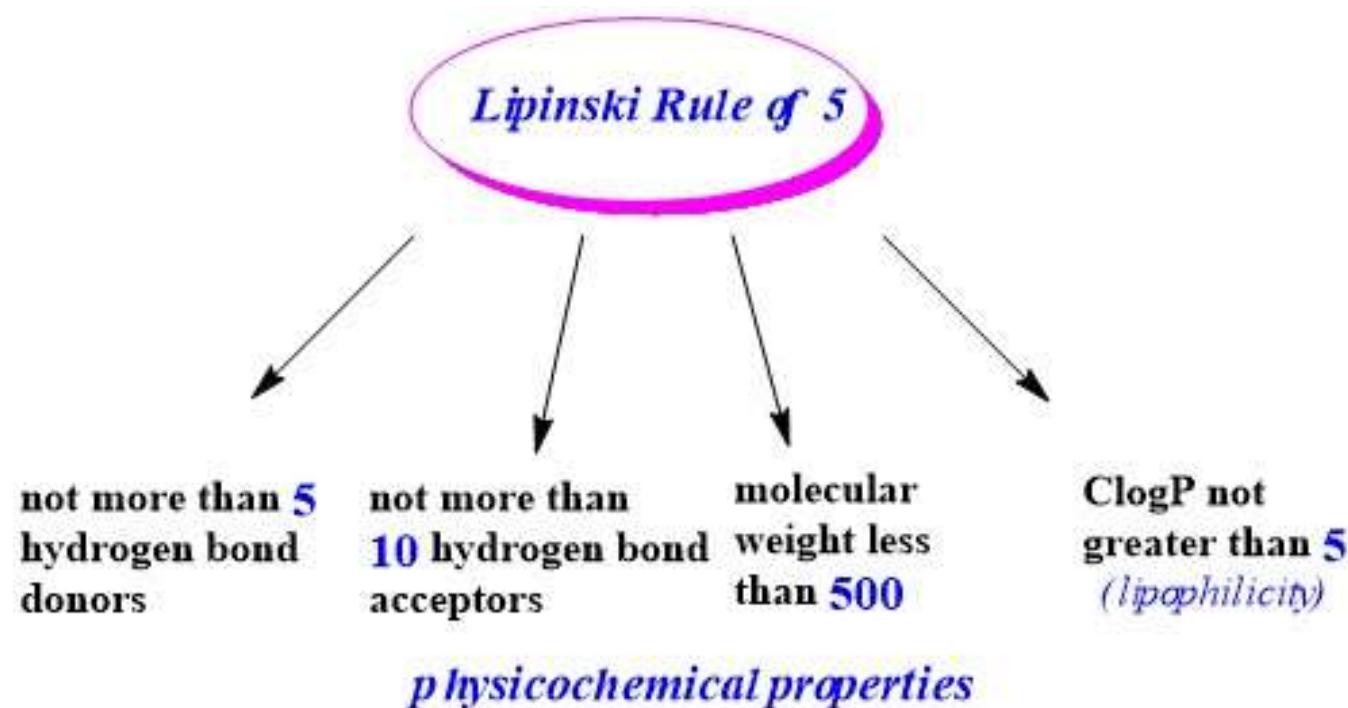
atenolol

Table 1. Predicted and experimental (in parentheses) ADME-related properties.

	Chlorpromazine	Atenolol
$\log S$	-4.5 (-5.01)	-0.61 (-1.30)
$\log P$	4.80 (5.19)	0.40 (0.16)
$\log \text{BB}$	0.74 (1.06)	-1.09
$\log K_{\text{hsa}}$	0.78 (1.10)	-0.79 (-0.48)
PCaco (nm/s)	2003	66 (33)
PMDCK (nm/s)	1425	33 (18)
CNS Activity	++	--

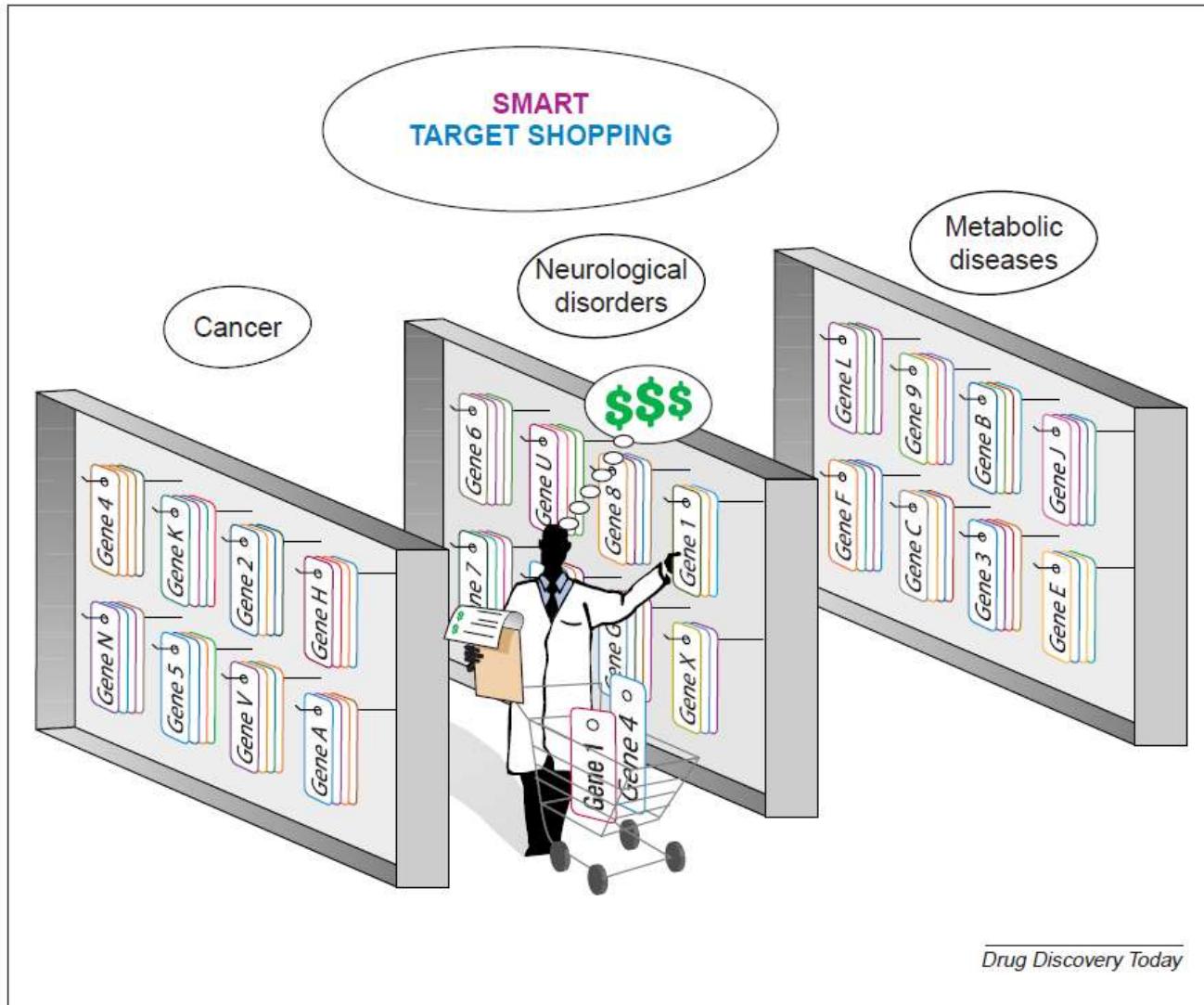
Lipinski rule of 5

Christopher Lipinski formulate this rule of thumb to determine if a pharmacologically active substance is likely to work as an *oral drug*.



Lipinski, CA (2000) "Drug-like properties and the causes of poor solubility and permeability"
J Pharm Tox Meth 44:235-239

Selecção de targets na era pós-genómica



Desenho de fármacos baseado em estrutura

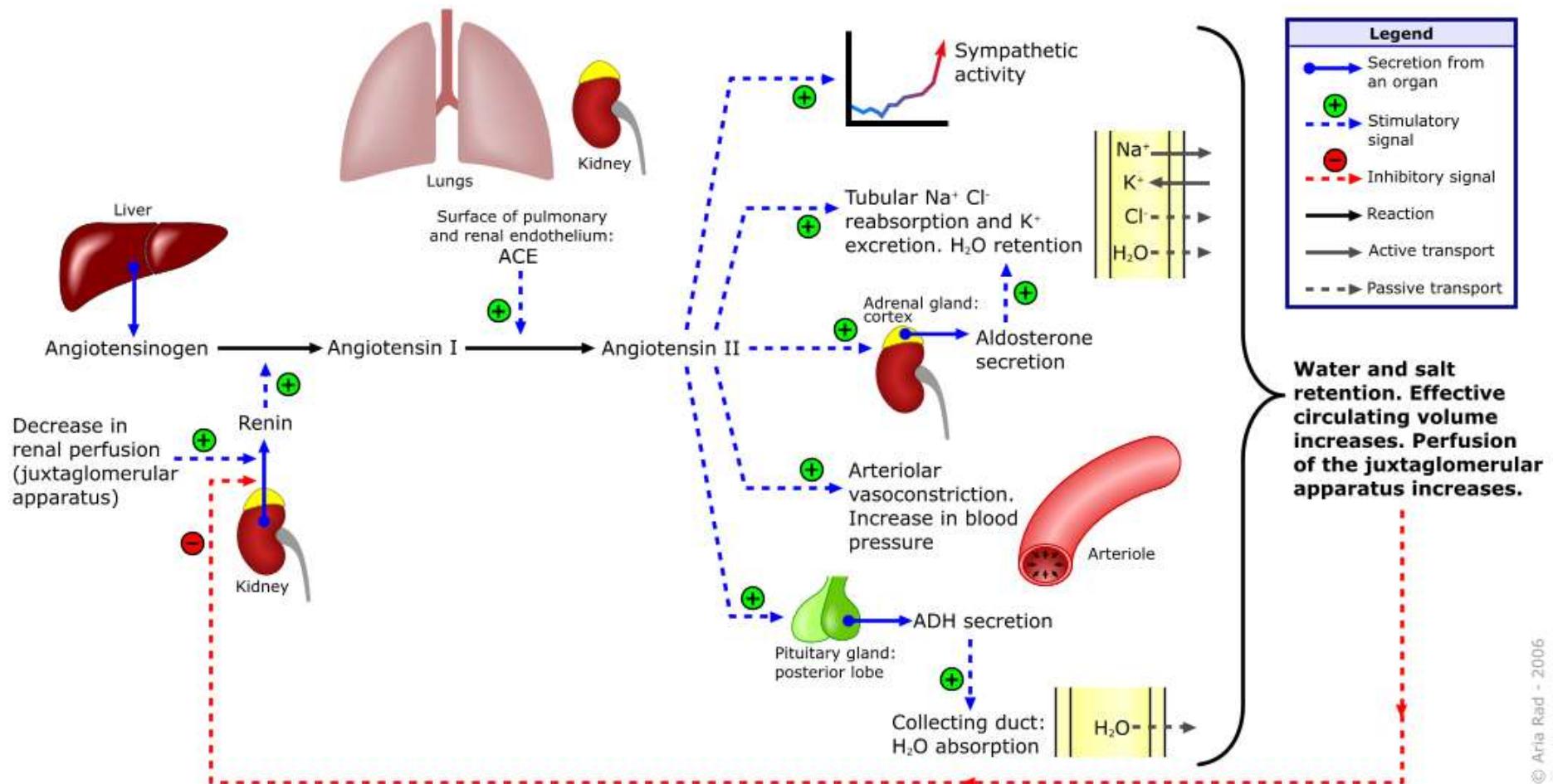
- O SBDD tornou-se possível com o desenvolvimento de uma vasta biblioteca de estruturas de receptores e enzimas
- Neste tipo de abordagem a forma e características electrónicas do centro activo são consideradas desde o início
- As estruturas cristalográficas da proteína e receptor são determinadas experimentalmente permite obter informação sobre as interacções do complexo
- Com base na informação estrutural procura-se encontrar as modificações que optimizem a interacção do ligando com o receptor
- Optimização da potência, afinidade e selectivida, preservando as propriedades ADMET !

Um exemplo de SBDD: desenvolvimento de inibidores da ACE



- O octapéptido Angiotensina II promove um aumento da tensão arterial
- Ferreira e Vane isolam um péptido do veneno da jararaca com capacidade de inibir a ACE
- Ondetti e Cushman reconhecem a similaride estrutural entre a ACE e a Carboxipeptidase A
- A estrutura da Carboxipeptidase A, bem conhecida na altura, serve de modelo à ACE
- O ácido benzil succínico é um inibidor potente da Carboxipeptidase A
- Os aminoacil-substituintes do ácido succínico revelaram-se inibidores potentes da ACE!

Angiotensin II : mechanisms of action



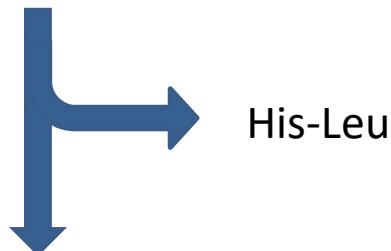
© Aria Rad - 2006

ACE also degrades the **blood-pressure-lowering nonapeptide bradykinin**

Angiotensina I

Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu

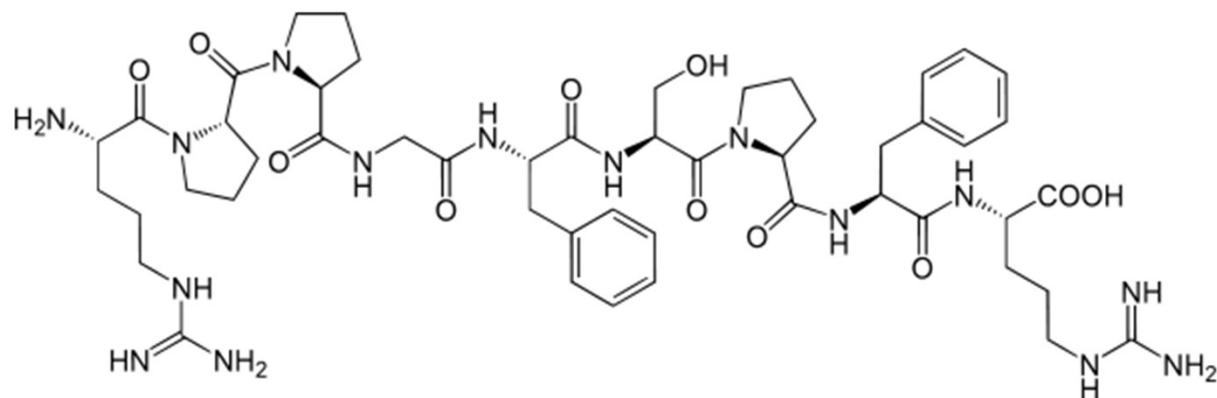
ACE (angiotensin-converting enzyme)



His-Leu

Angiotensina II

Asp-Arg-Val-Tyr-Ile-His-Pro-Phe



Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg

Bradicinina

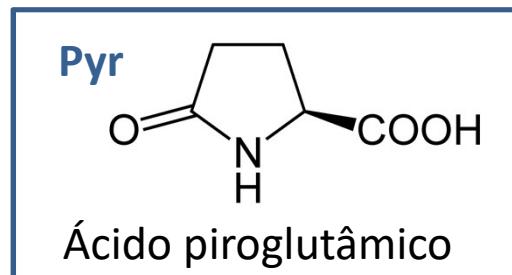
Descoberta do teprótido

Em 1965 Sérgio Ferreira e Joseph Vane isolam do veneno da cobra brasileira jararaca uma mistura peptídica capaz de prolongar o efeito anti-hipertensivo da bradicinina por inibição da sua degradação. Desta mistura foi isolado o péptido **teprótido**, de fórmula:



Foi posteriormente demonstrado que este péptido inibe também a formação de Angiotensina II a partir da Angiotensina I.

Miguel Ondetti, da empre Squibbs, sintetizou este péptido o qual se verificou se um potente inibidor da ACE ($K_i = 100 \text{ nM}$), tanto em animais como humanos, embora não possa ser usado como fármaco - os péptidos não são geralmente disponíveis por via oral.

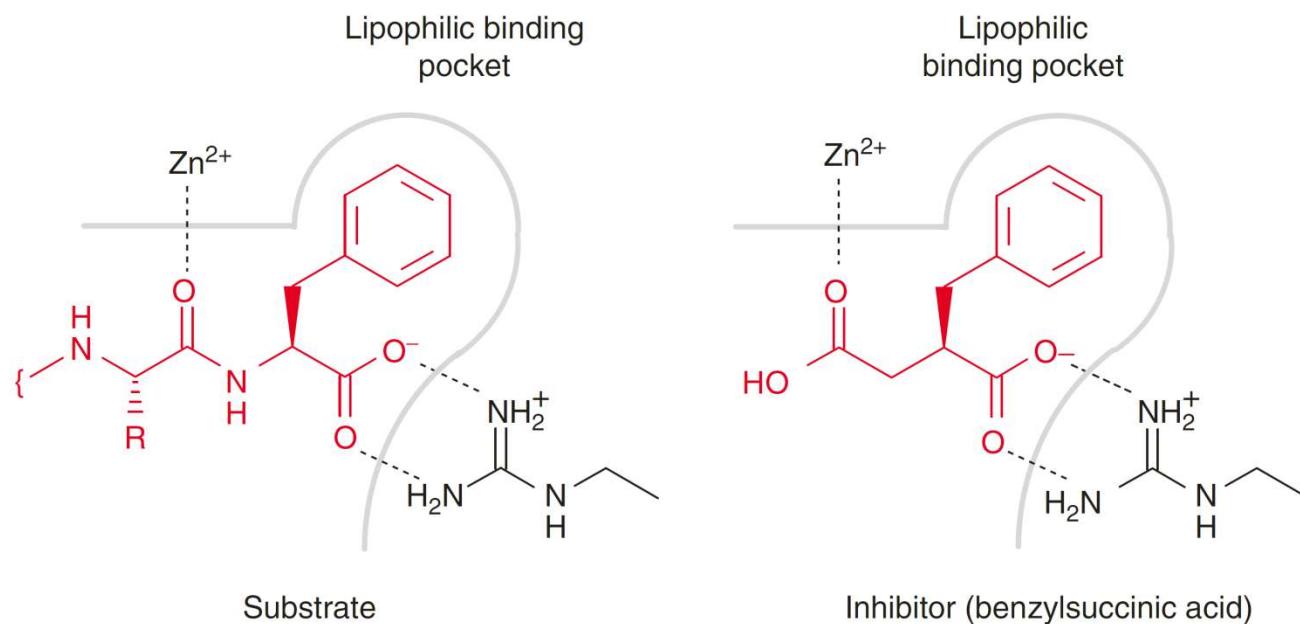


Jararaca (*Bothrops jararaca*)

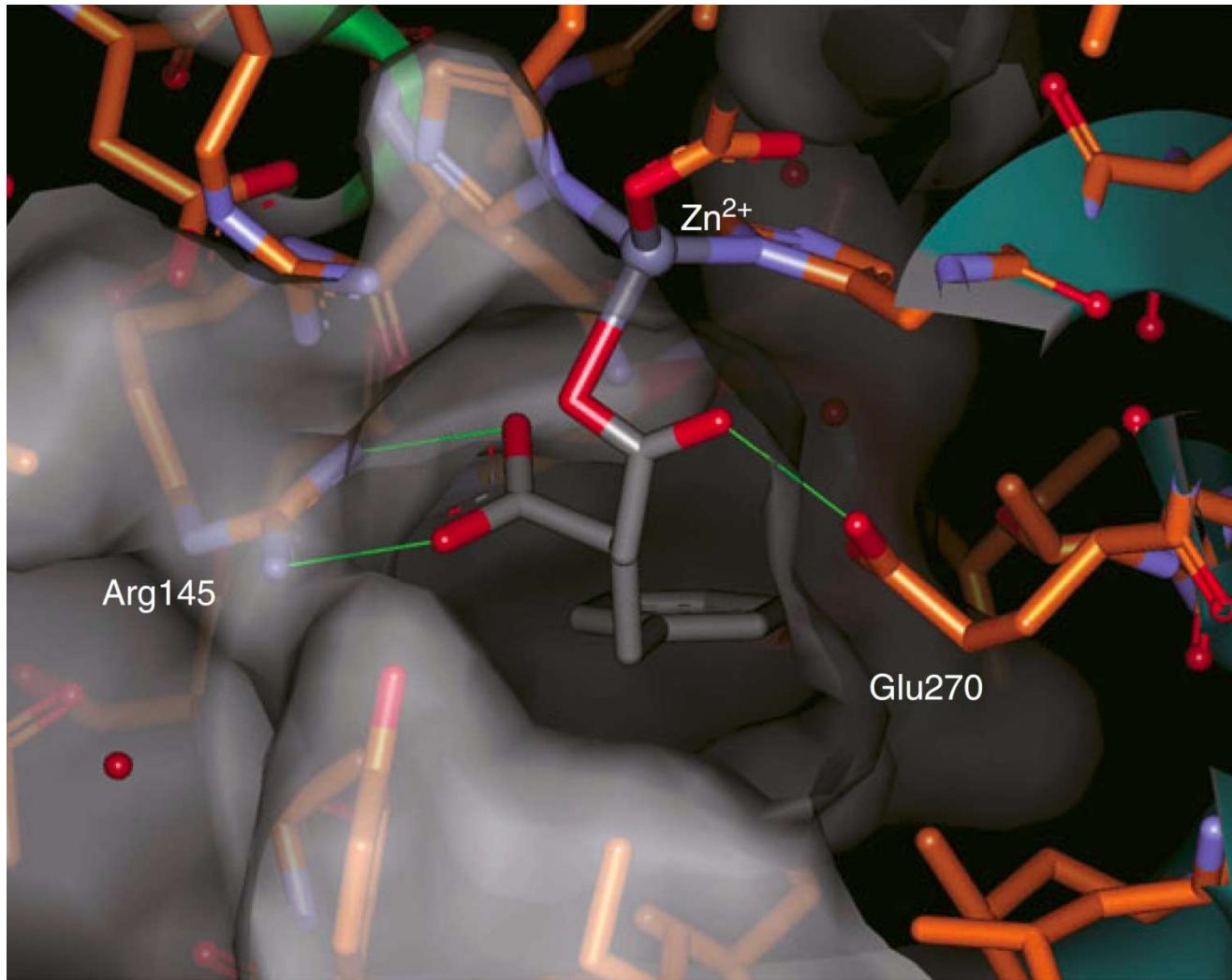
Carboxypeptidase A versus ACE

Nos anos 70 Miguel Ondetti e David Cushman (Squibb) reconhecem a similaridade de mecanismo entre a ACE e *carboxypeptidase A*, uma enzima que remove aminoácidos C-terminais de uma cadeia polipeptídica.

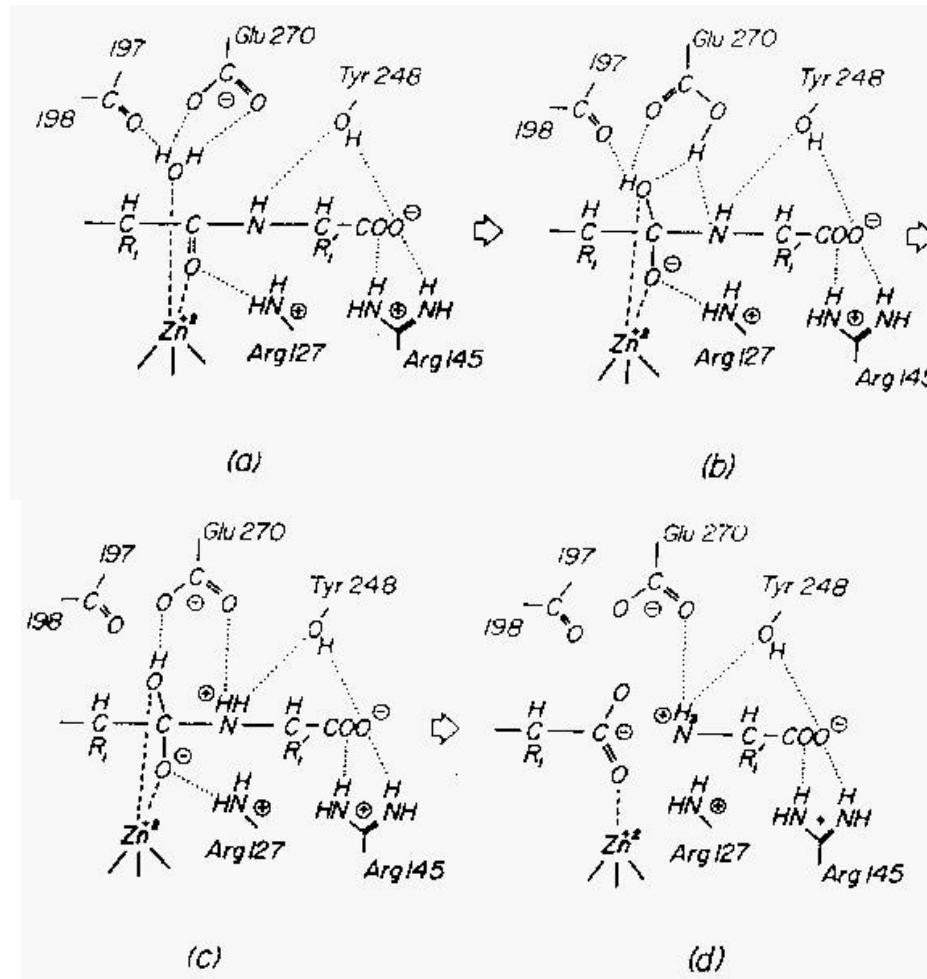
O ácido benzil-succínico é um potente inibidor da Carboxypeptidase A, funcionando com um análogo da região C-terminal do substrato.



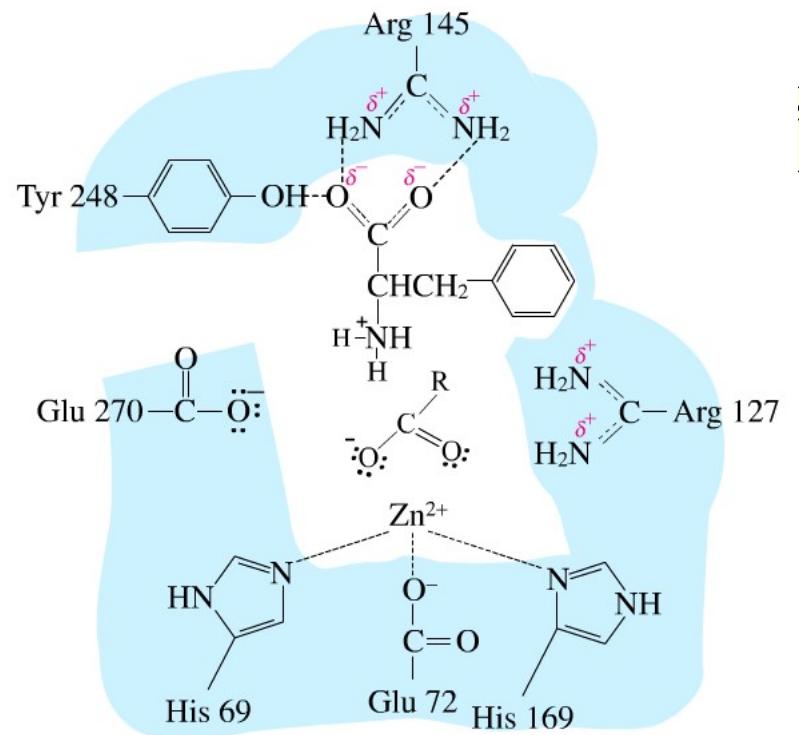
Carboxypeptidase A em complexo com o inibidor benzil succinato



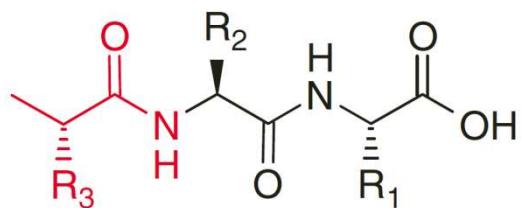
Mecanismo catalítico da Carboxipeptidase A



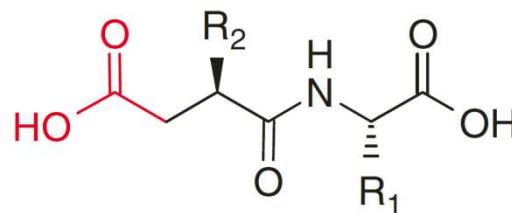
Mecanismo catalítico da Carboxipeptidase A



Dado que a ACE remove os *dois* resíduos C-terminais, Ondetti e Cushman investigaram a possibilidade usar derivados amino-substituídos do ácido succínico como inibidores desta enzima.

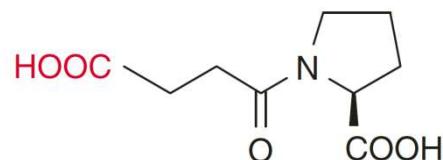


Substrate



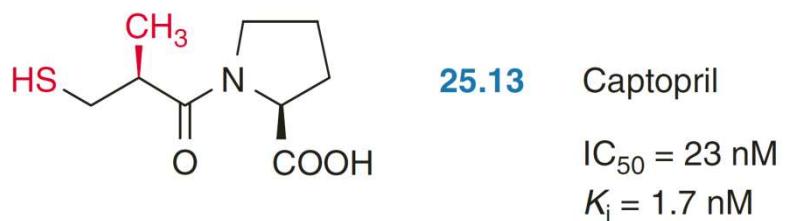
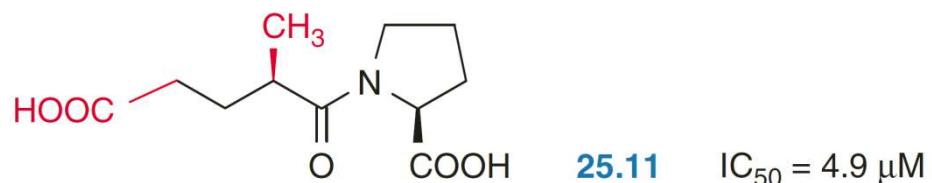
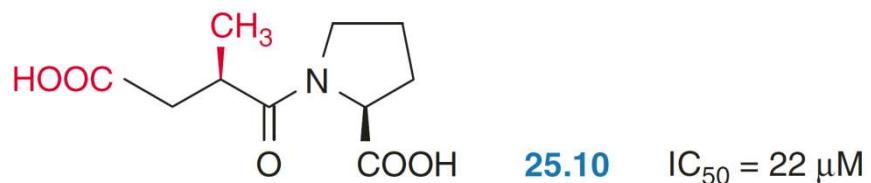
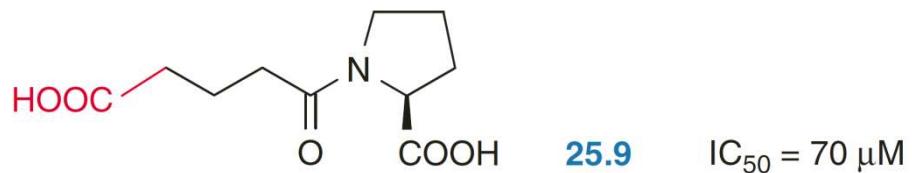
Inhibitor

Ala-Pro ($K_i = 230 \text{ mM}$)



Succinil-L-prolina ($IC_{50} = 330 \mu\text{M}$)

Optimização do lead





25.14

$IC_{50} = 17 \mu M$



25.15

$IC_{50} = 4300 \mu M$



25.16

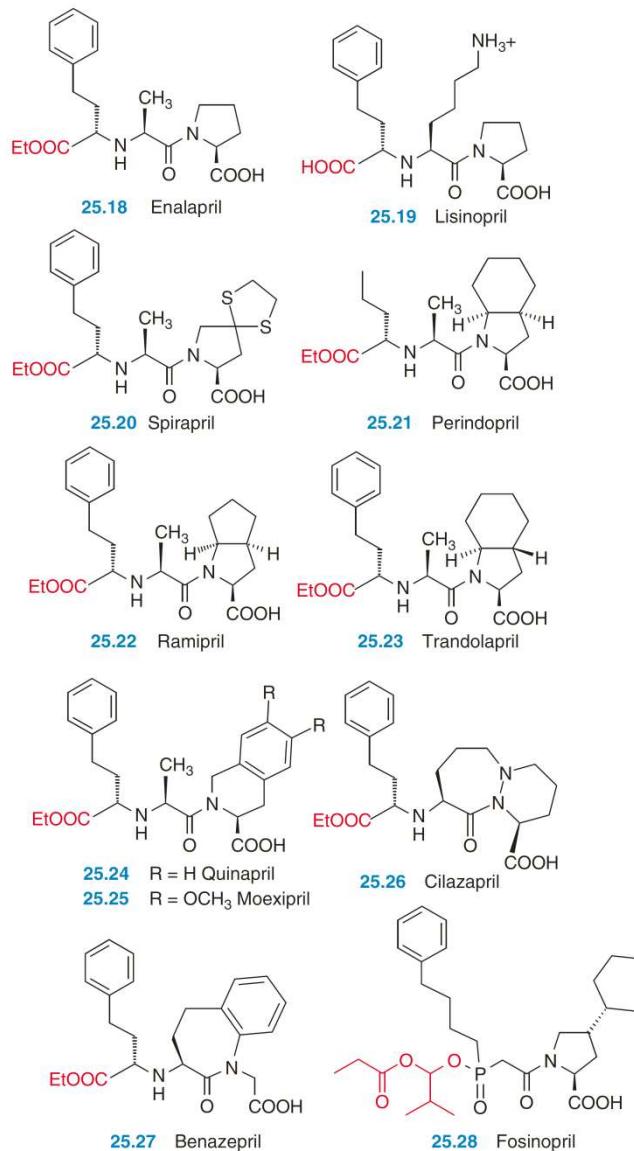
$IC_{50} = 2.8 \mu M$



25.17

$IC_{50} = 1100 \mu M$

Estudos com estes compostos indicaram a necessidade do grupo tiol, grupo carboxílico livres e da presença do anel de prolina.

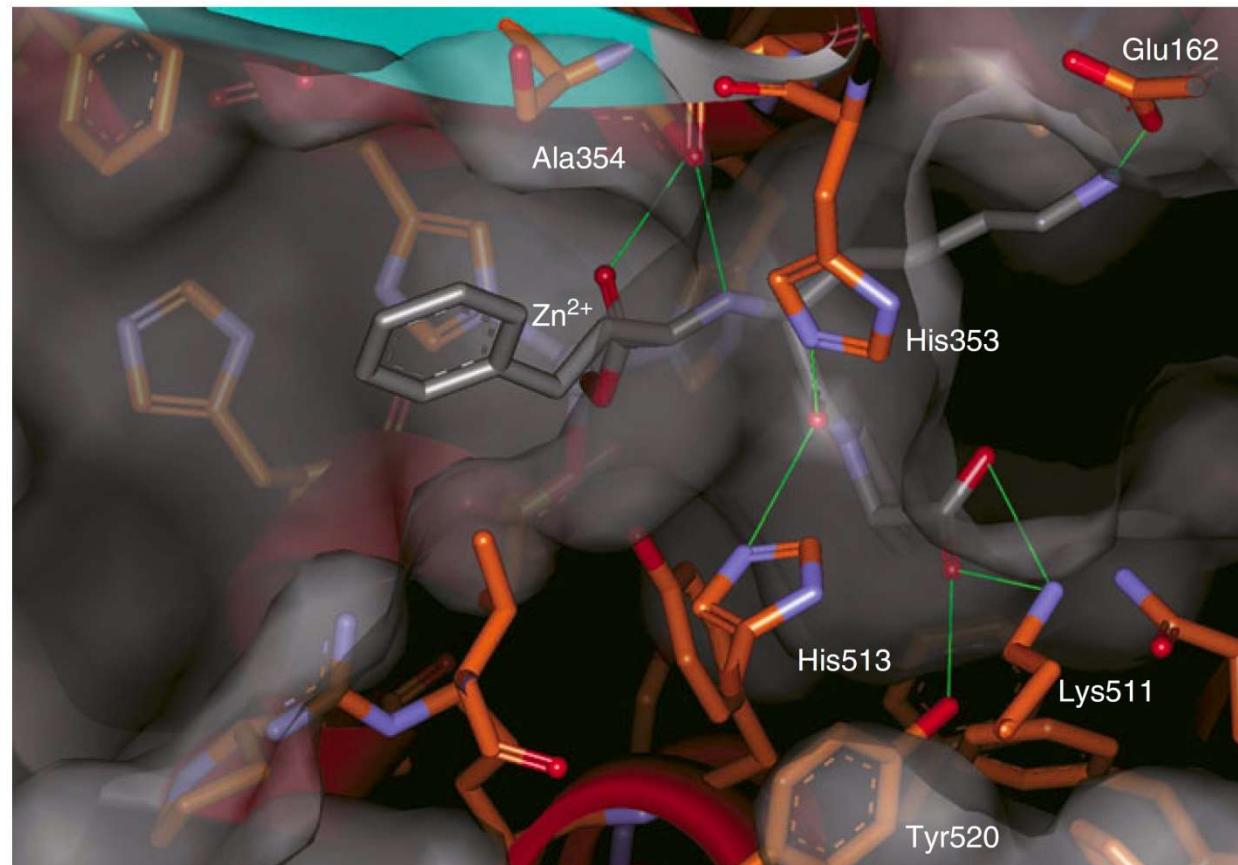


Outros inibidores da ACE desenvolvidos posteriormente.

The studies described above exemplify the great heuristic value of an active-site model in the design of inhibitors, even when such a model is a hypothetical one. Only when suitable information on substrate specificity and mechanism of action of an enzyme is available can one make a reasonable working hypothesis with regard to complementary functionality needed in an inhibitor.

-- David Cushman

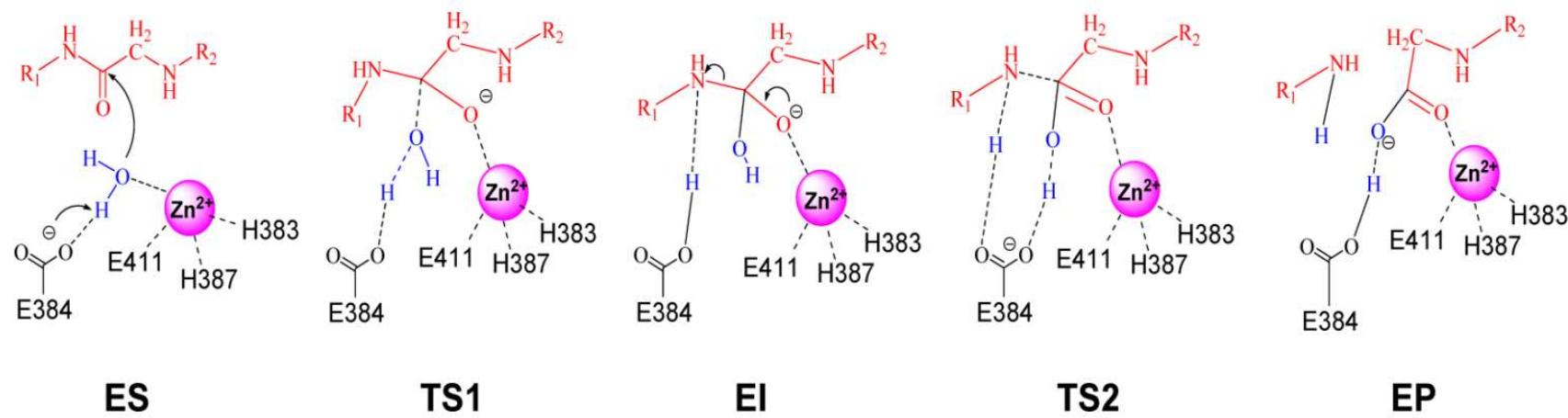
Em 2003 o grupo de Edward Sturrock determinou a estrutura cristalográfica da ACE, mostrando a presença de dois domínios com actividade catalítica (domínio N-terminal e domínio C-terminal) e sequências diferentes (60% de identidade). Os centros activos têm actividade diferente na degradação da bradicinina, que pode assim ser desacoplada parcialmente do efeito sobre a angiotensina I.



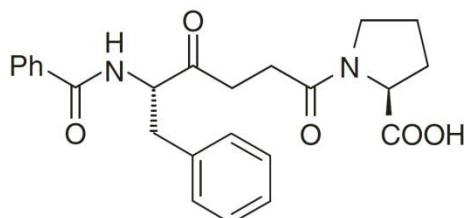
t-ACE em complexo com o lisinopril

Natesh *et al.* (2003) Nature **421**:551

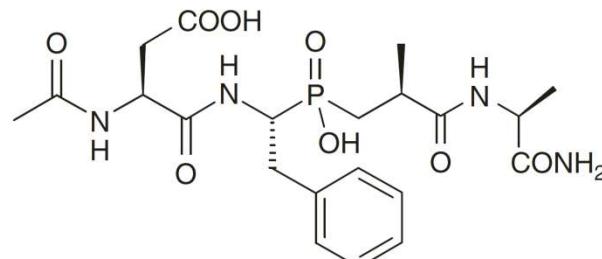
Mecanismo catalítico da ACE (hipotético)



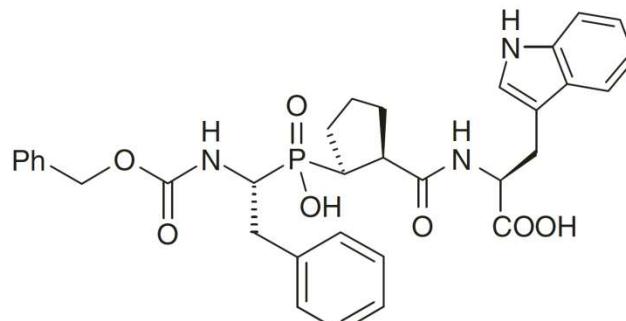
Inibidores específicos para os domínios N e C da ACE



25.29 Keto-ACE



25.30 RXP407



25.31 RXPA380

Compound	N-domain inhibition (nM)	C-domain inhibition (nM)
RXP A380 25.31	10,000	3.0
Captopril 25.13 ^a	8.9	14.0
Enalapril 25.18 ^b	26.0	6.3
RXP407 25.30	2.0	2,500
Lisinopril 25.19 ^b	44.0	2.4
Keto-ACE 25.29	15,000	40.0