DRUG DISCOVERY TODAY : STRATEGIES AND TECHNOLOGIES

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1- MEDICINAL CHEMISTRY

11 _ DEFINITION AND OBJECTIVES

Definition (IUPAC) :

« **Medicical Chemistry** concern the **discovery**, the **development** and the interpretation of mode of action of biologically active compounds **at the molecular level**. Emphasis is put on drugs, but the interests of the medicinal chemist are not restricted to drugs and **included bioactive compounds in general**. Medicinal chemistry is also concerned with the study, identification and synthesis of the **metabolic products of these drugs and related compounds** ».

Interdisciplinary science covering a wide domain with an interface situation :

Organic Chemistry

- Biochemistry
- Molecular biology
- Immunology
- Genetics

-Physical chemistry
- Crystallography
- Spectroscopy
- Computer assisted design and techniques of simulation
- Data analysis and visualization

12 _ DRUGS AND DRUGS SUBSTANCES

WHAT IS A DRUG ?

Drug (D) = **Drug substance** (also named Active Pharmaceutical Ingredient, API) + **Excipients** (Ancillary substances, Es).

API + Es —**Formulation**→ **D**

(galenics = pharmaceutical technologies)

WHAT MAKES A CHEMICAL « DRUGGABLE » ?

The ideal profile of a new drug substance is defined as following :

- **new chemical entity** for potent activity ant registration ;
- **maximum four-steps synthesis** (with, for example, no heavy metal catalyst and no environmentally problematic waste);
- **stable up to 70° C** (even in humid air and toward light) ;
- **solide-state properties** (crystalline, not polymorphous, not hygroscopic), that make it a perfect partner for compaction ;
- **oral bioavailability > 90%** (with no interindividual variations) :
- **very high activity** and pharmacokinetic profile, enable once a day-dosage at 5 - 10 mg/kg.

WHAT IS LIPINSKY'S RULE OF FIVE ?

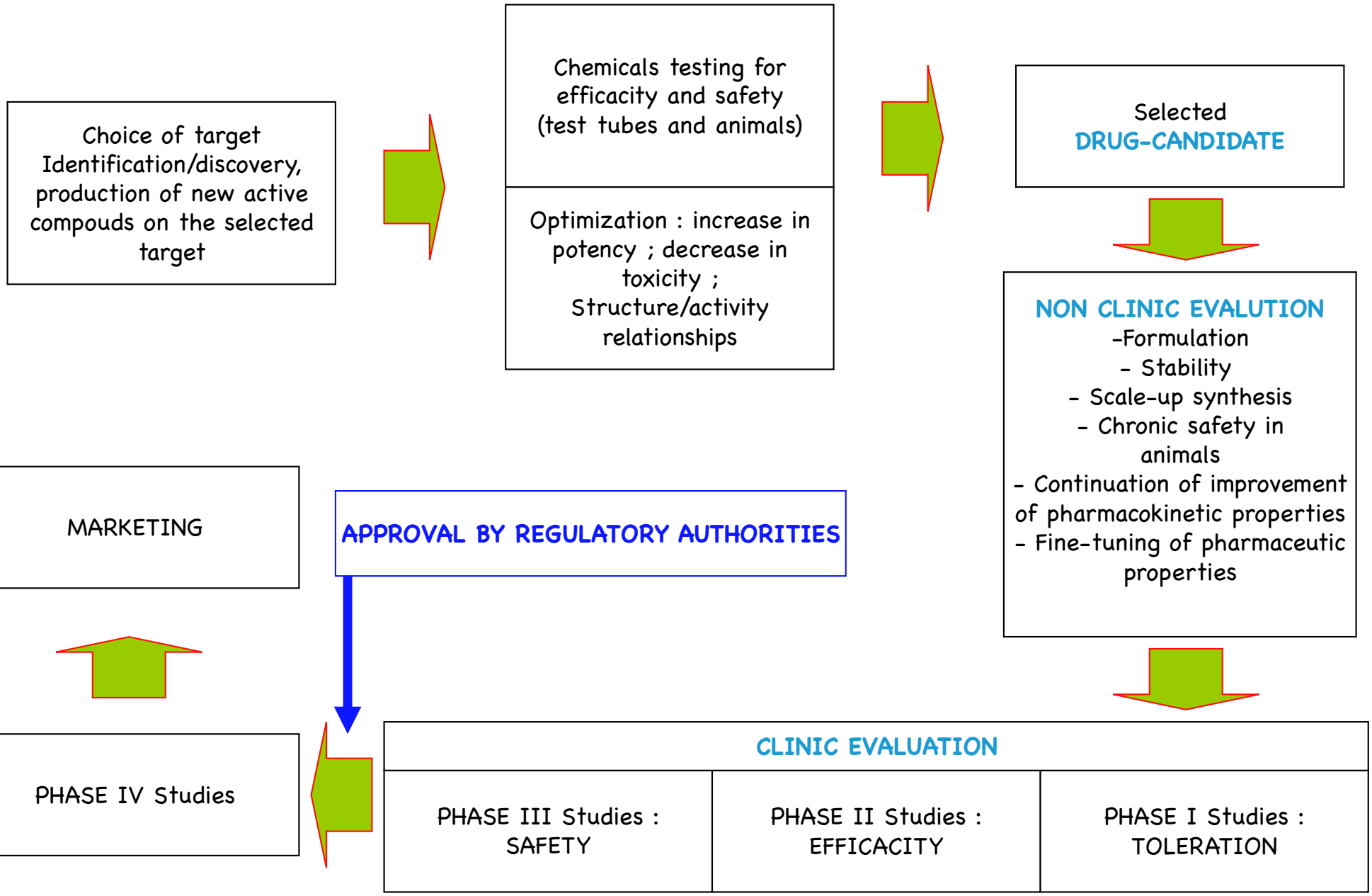
A very important tool for drug development, to :

- evaluate druglikeness ;
- determine if a chemical compound with a certain pharmacological or biological activity has properties that would make it likely oral active drug in humans.

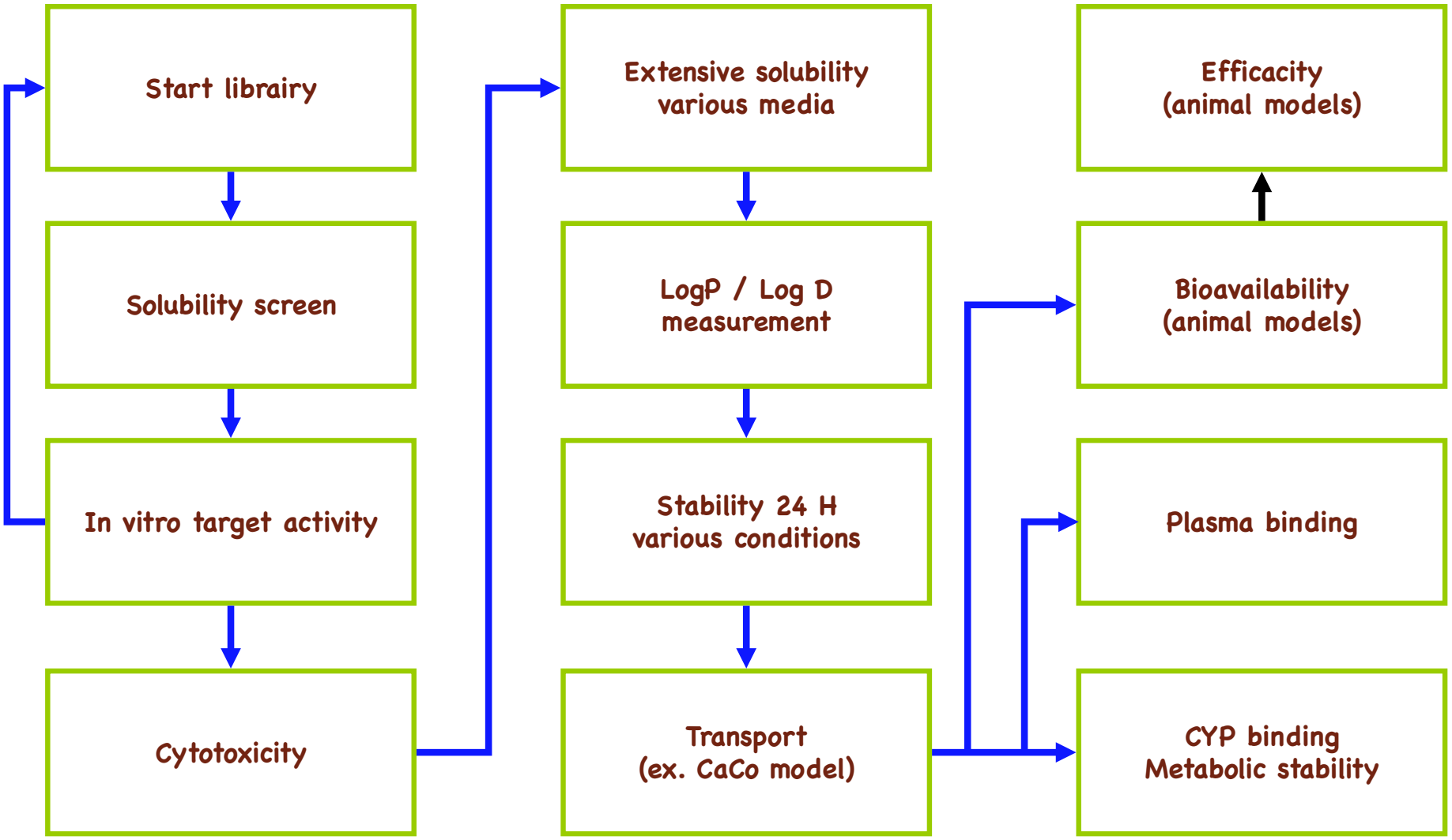
In general, an orally activ drug has **no more than one violation of the following criteria** :

- **not more than 5 hydrogen bond donnors** (N, O, with one or more hydrogen atoms) ;
- **not more than (5 x 2) hydrogen bond acceptors** (N or O) ;
- a molecular mass **not greater than 500 daltons** ;
- an octanol-water partition coefficient, **logP, not greater than 5**.

13 _ STEPS OF DRUG DEVELOPMENT



Example 9 : an example of an optimization algorithm :



14 _ MOLECULAR DRUG TARGETS

DRUG TARGET AND RESPONSE OF THE ORGANISM :

- Drugs act by **increasing or decreasing a normal function, ; not endow the organism with new functions** (except for gene therapy) ;

- Vast majority of drugs produce their effects by **interaction with proteins** :

  - on the surfce of the cell (plasma membrane) : receptors, ionic channels, transporters ;
  - on the interior of the cell : enzymes, nuclear receptors.

- Some others act **extracellularly**, at non cellular contituents (ex. : neutralization of gastric acid, ...) ;

- DNA is also a possible target ;

- **Miscellaneous mechanisms** : = non-specific consequences of chemical properties of the drugs (detergents, alcohol, oxidizing agents, ...) ;

- **Acceptor sites** : interactions between drugs and the biological components may occur. For example, binding of APIs to plasma albumin, with major consequences on the transport in blood circulation to organs, and on the grug action or its rate of action.
  So, albumin is considered as an acceptor site rather than a target or a receptor.

DRUG BINDING :

Various physicochemical interactions exists betwen a ligand and the target to establish the target-drug interaction :

- **Hydrophobic interactions** : important role in stabilizing the conformation of protein and in the association of hydrophobic structures between drug and targets.

- **Hydrogen bonding** has a considerable importance both in the maintaining of secondary and tertiary structure of the target and in the target-drug interaction.

- **Charge transfert complexes**, formed betwen electron-rich donnor molecules and electron-deficient acceptors.

- **Ionic bonds** : important in the action of ionizable drugs ; most targets have a number of ionizable groups as COO^- or NH_3^+ at physiological pH.

- **Covalent bonds** : resulting in the formation of a long-lasting complex. Less important in drug-target interaction because it's often necessary to need readily reversible interactions.

VARIOUS LIGANDS :

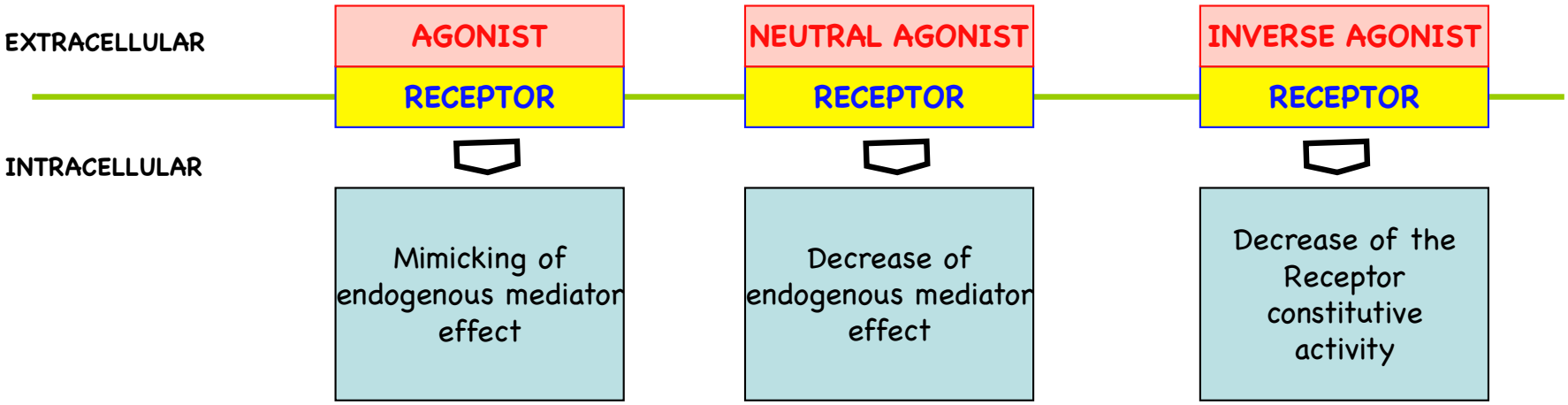
Terms used to characterize different ligand types differ accordind to the biochemical nature of the targets.

- **Enzyme ligands** : More often, the resulting effect required consists in an **inhibition** of the enzyme activity, binding the **active site** (competitive inhibitors) or to **allosteric sites** (non-competitive or allosteric inhibitors).
  Actiivation of an enzyme is more difficult, but some drugd are known to activate enzyme by direct binding ex. : (adenylcyclase by farskolin).

- **Membrane transporters and ion channels** : their permeability can be increased or decreased by direct binding of **openers** or **blockers**..

- **Receptors of mediators** are able to interact with a large diversity of ligands types.

Example 10 : various effects resulting of ligand-receptor interactions :



2- LEAD COMPOUND DISCOVERY STRATEGIES

21 _ DEFINITIONS : HITS, LEADS and DRUG CANDIDATES

Hit = active substance having a preferential activity for the target, and satisfying the following criteria :

- Reproductible activity in a relevant bioassay ;
- Confirmed structure and purity ;
- Specificity for the target under study ;
- Chemically tractable structure.

Identification of Hits => screening of a wide range of structurally diverses small molecules in an *in vitro* bioassay.

Requirements : Confirmation and validation of activity. For validation, typical criterias are :

- Activity *in vivo* ;
- Not display hERG toxicity ;
- Display clear SAR (hit and analogs) ;
- Not contain chemically reactive functions ;
- Must provide patent opportunities.

Only then, Hit becomes a '**Lead**' (or, also, a '**Lead substance**').

If a lead **emerge** from additionnal **studies on ADMET**, it acquires the '**Drug-candidate**' status.

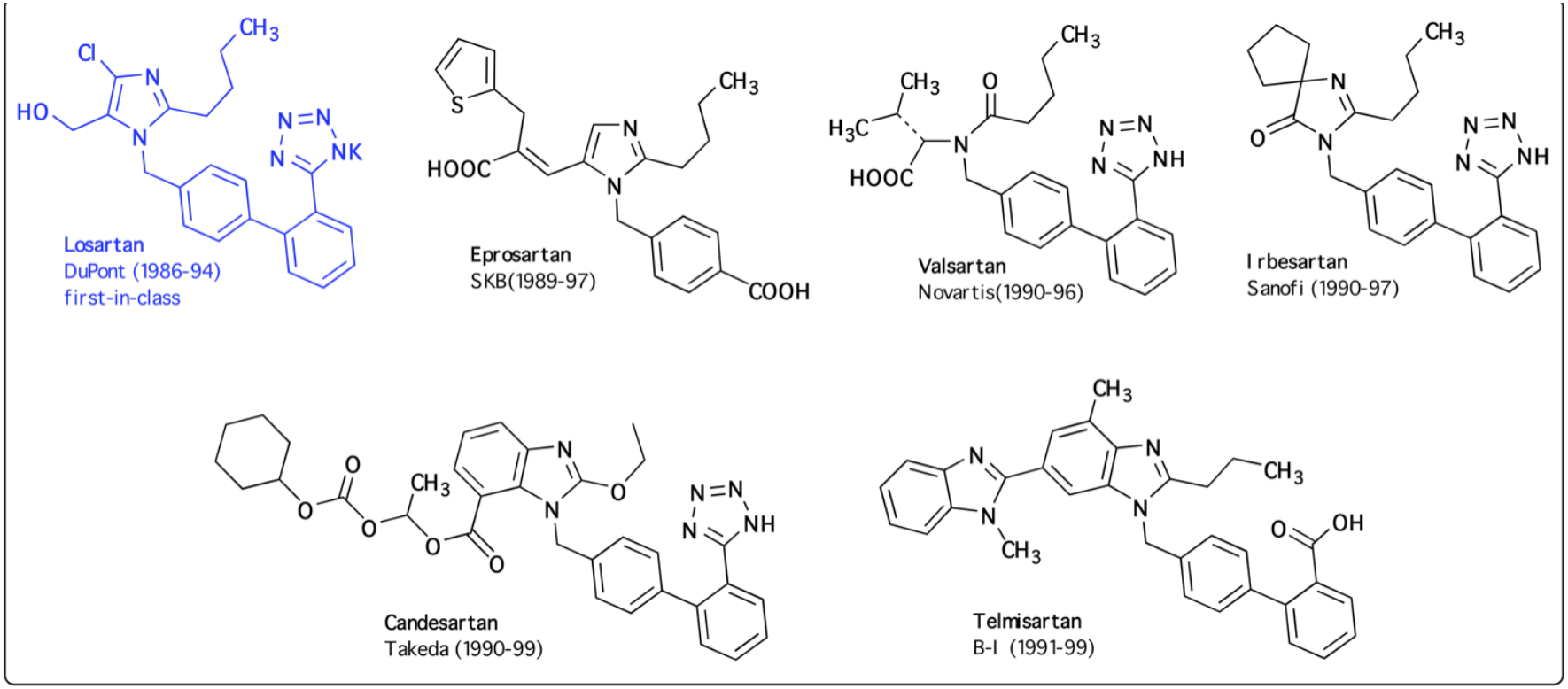
22 _ ANALOG DESIGN

Design and synthesis of analogs of existing actives compounds :

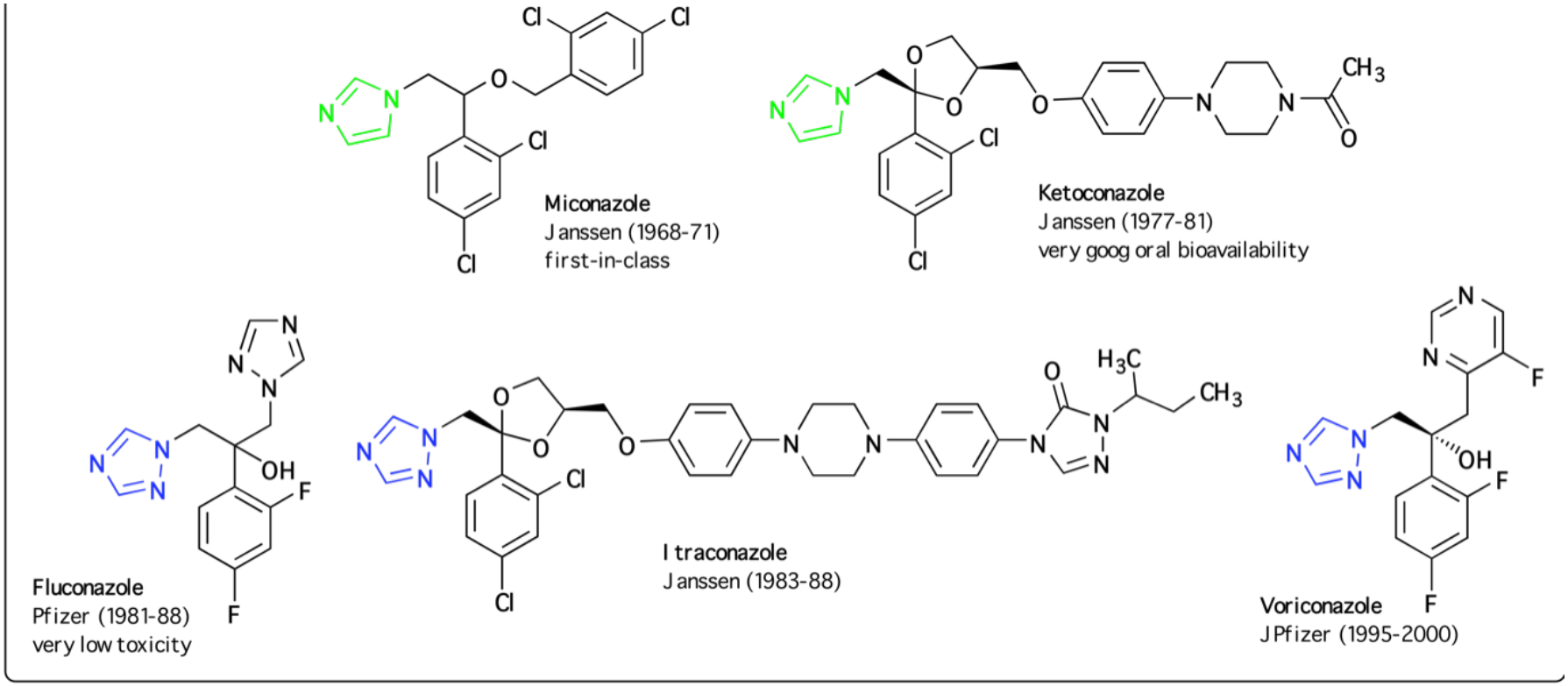
Start with known active principles and, using various chemical transformations, prepare new compounds with :

- Increase of potency ;
- Better specific profile ;
- Improved safety.

Example 1 : Sartans, Angiotensin II Receptor Antagonists (ARA) :



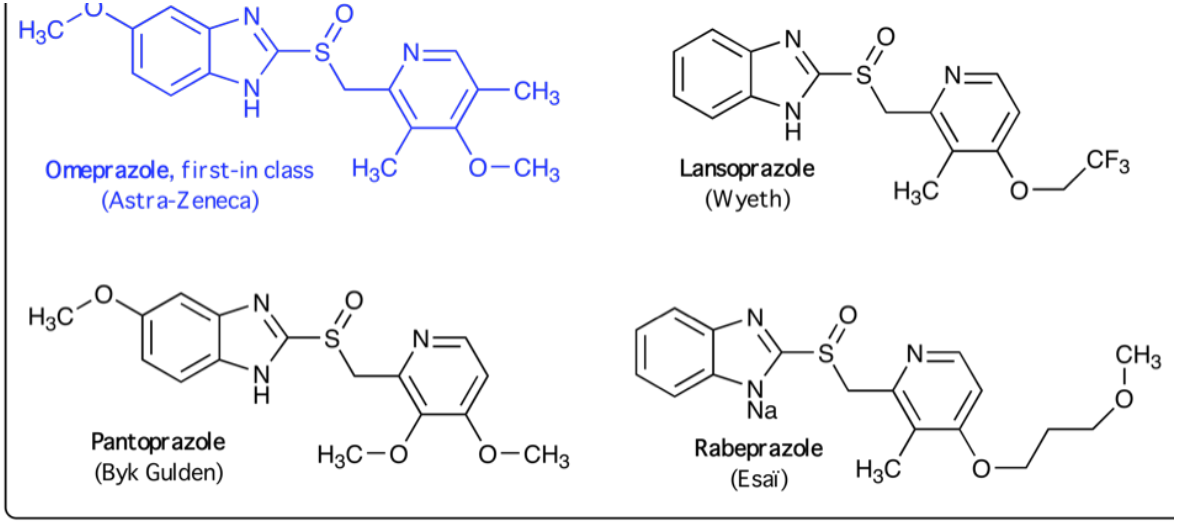
Example 2 : Conazoles, fungistatics :



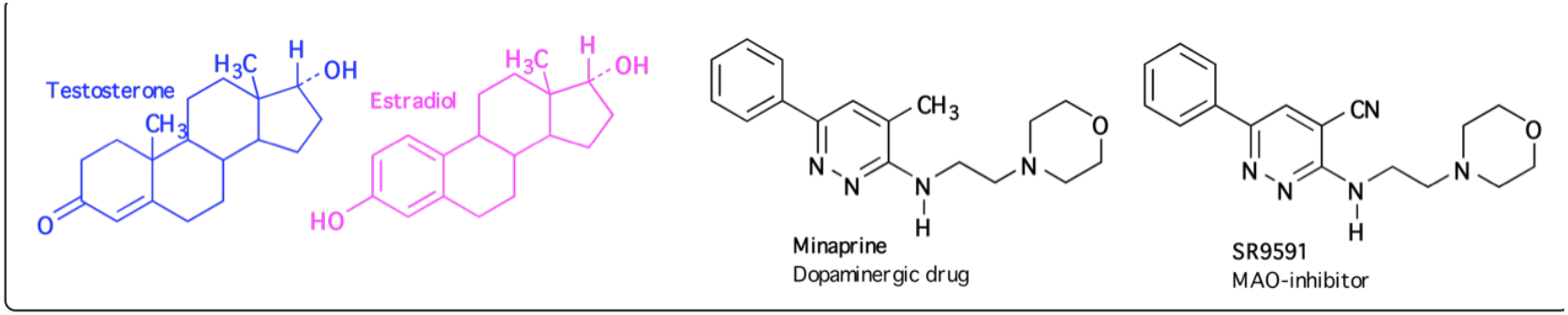
Three categories of analogs :

- Presenting chemical and pharmacological similarity (= mee-too compounds) ;
- Presenting only chemical similarity (observation of emergent activity) ;
- Displaying similar pharmacological properties but with totally different chemical structure.

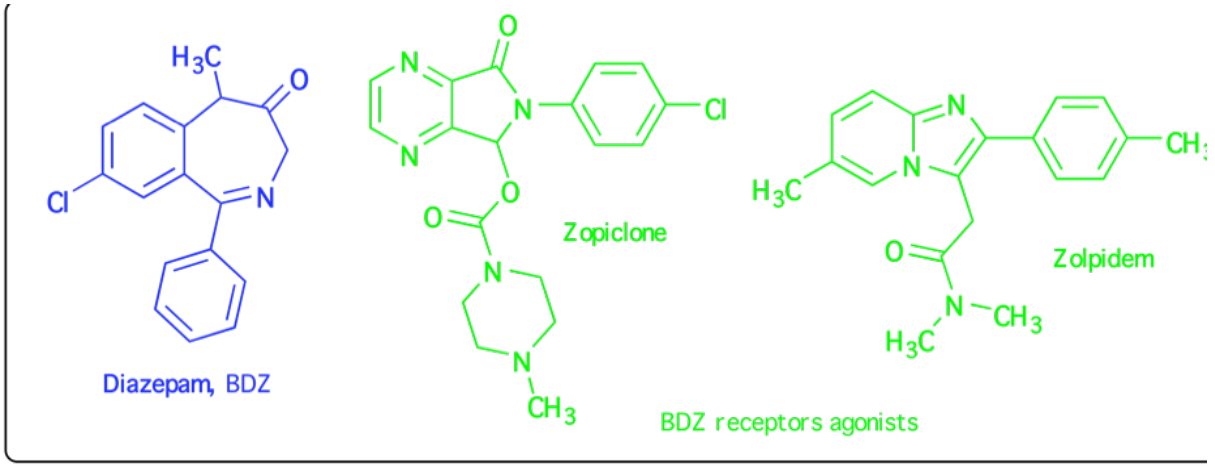
Example 3 : Me-too development in Proton-Pump inhibitors class :

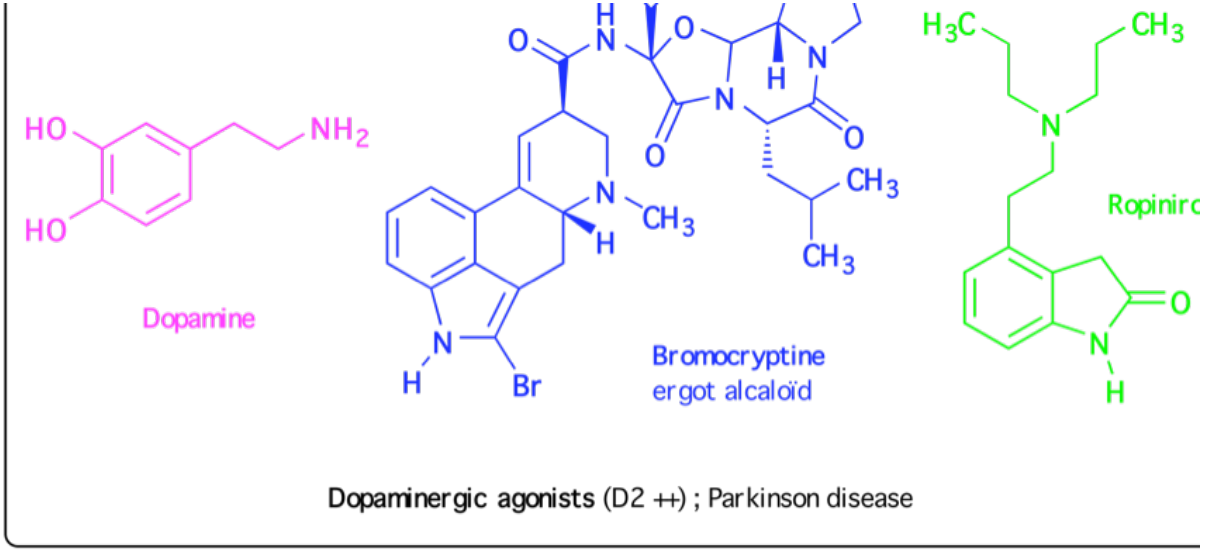


Example 4 : Chemical similarity, pharmacological difference :



Example 5 : Chemical difference, pharmacological similarity :





23 _ SYSTEMATIC SCREENING

Consists in screening of new molecules (synthetic or natural origin) **on an animal model or on any biological test, without having in mind the hypotheses on its pharmacological or therapeutic potential.**

In practice, systematic screening can be achieved in **five different manners :**

- **Extensive Screening** = application to a small number of chemically sophisticated and original compounds of a very exhaustive pharmacological investigation ;

- **Random Screening**, on the contrary, strive to find, among a great number of compounds (hundreds or thousands) one that could be active in a given indication ;

- **High-Throughput Screening** : with the arrival of robotics and miniaturization of in vitro testing methods, it became possible to screen thousands of compounds on a large number of biological targets ;

- **Screening of synthesis intermediates** : as it's not excluded that synthesis intermediates shore pharmacological properties, it is always prudent to submit them to a biological evaluation ;

- **SOSA Approach** = Selective Optimization of Side Activities. Process in two steps :

  - screening of well-known drugs on newly identified pharmacological targets (limited approx. 1000 cpds) ;
  - optimization of hits in order to increase the affinity for the new target, and decrease the affinity for others.

24 _ EXPLOITATION OF BIOLOGICAL INFORMATION

A major contribution to the drug discovery of new drugs-candidates comes from the exploitation of biological informations :

- **Made in humans**, as :

  - Study of indigenous medicines (ethnopharmacology) ;

  - Clinical observations of side-effects ;
    ex.: sedative effect of **promethazine** => chlorpromazine, prototype of neuroleptics.

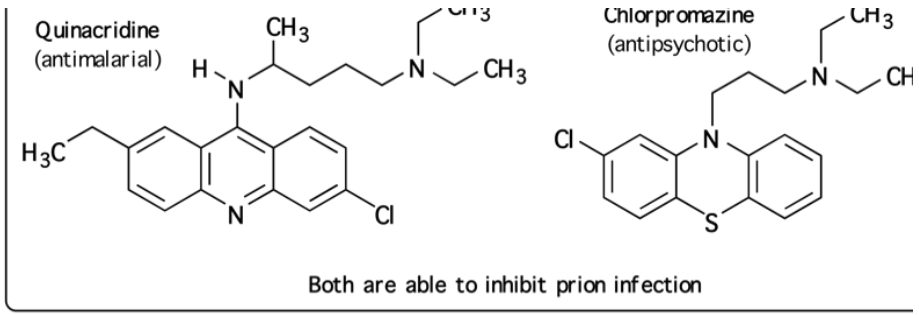
  - New uses for old drugs ;
    ex.: **thalidomide**, initially sedative/hypnotic drug with a very high teratogenic activity found a new use as immunomodulator.

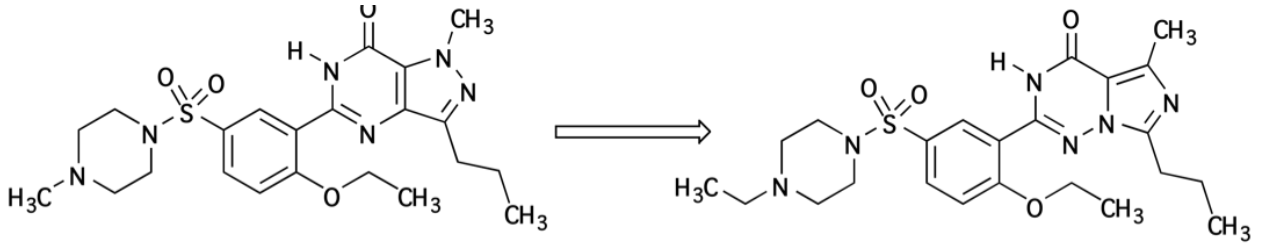
  - Fortuitous discovery of activites of pharmaceutical or industrial chemical products ;
    ex.: **nitroglycerin** (strong vasodilatating properties =>toxic manifestations during its Industrial manufacture) is used in angina pectoris and as cerebral vasodilatator (and others nitric esters, so).

- **Made in animals.**

- **Made in the plant kingdom and in microbiology.**
  ex.: antibiotics, anticancer agents, ….

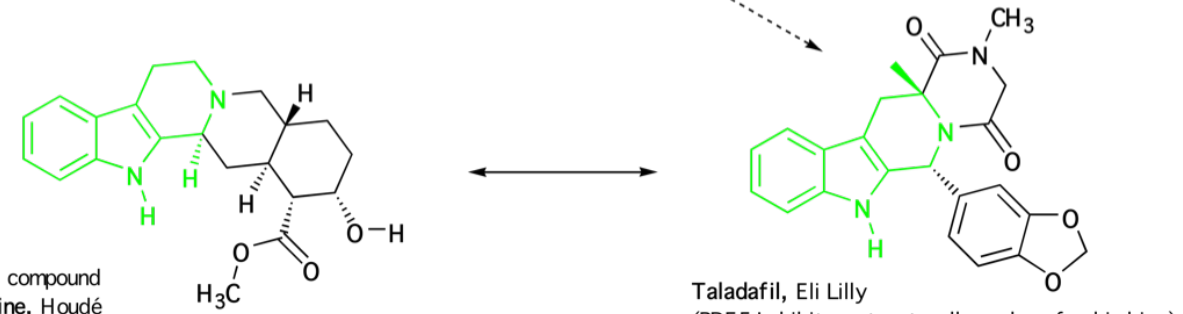
Example 6 : New use for old drugs :





Sildenafil, Pfizer
(PDE5 Inhibitor)
Brought to the clinic as hypotensive and cardiotonic drug ;
Today usefullness in erectile dysfunction.

Vardenafil, Bayer
(PDE5 Inhibitor, "me-too")



Natural compound
Yohimbine, Houdé
(structurally analog of Taladafil, but with α-2 and α-1 adrenergic receptors agonist activity, ant not PDE5 Inhibitor)

Taladafil, Eli Lilly
(PDE5 Inhibitor, structurally analog of yohimbine)

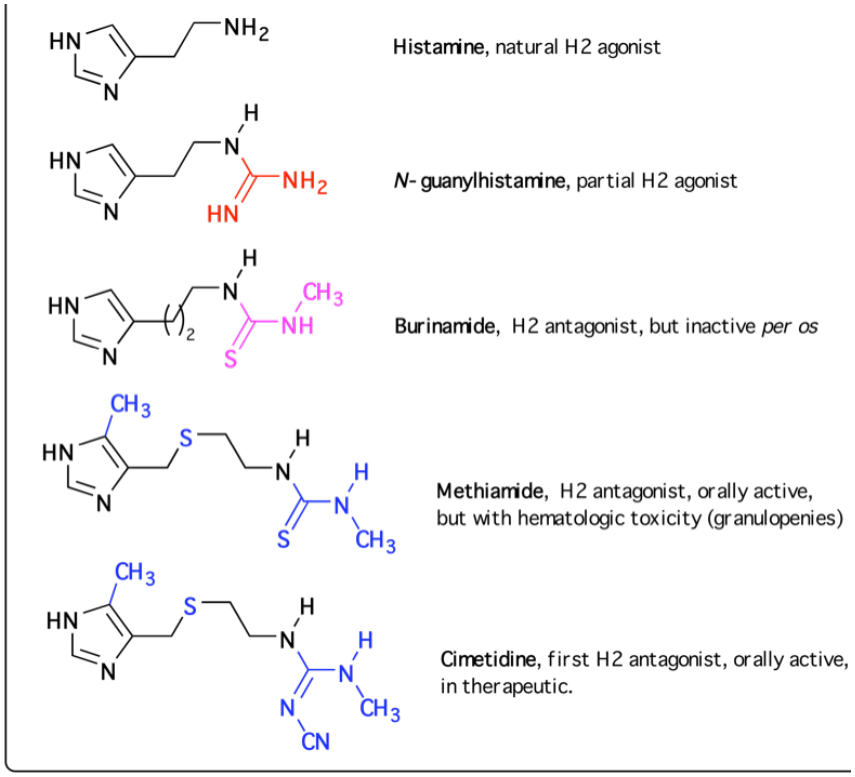
25 _ PLANNED RESEARCH AND RATIONAL APPROACHES

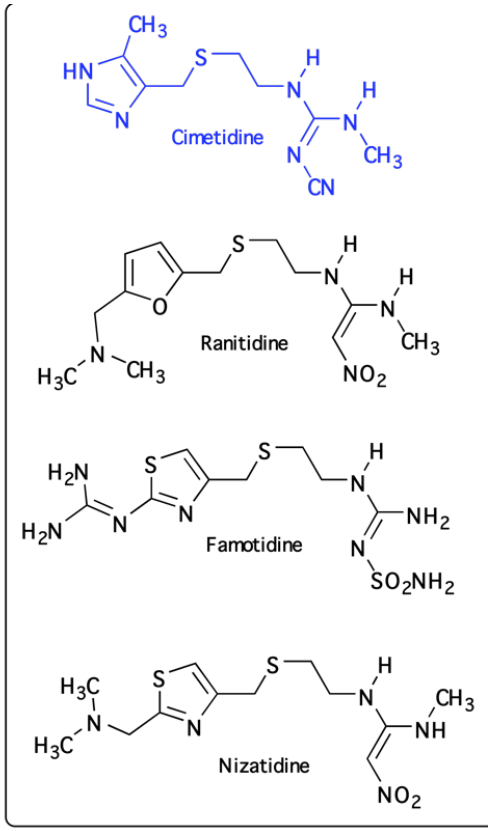
The most scientific approach ; based on the knowledge of the incriminated molecular target.

In first, the **choice of a therapeutic target** is necessary. Then, come identification/discovery, synthesis and biological evaluation of new actives substances interacting with the selected target.

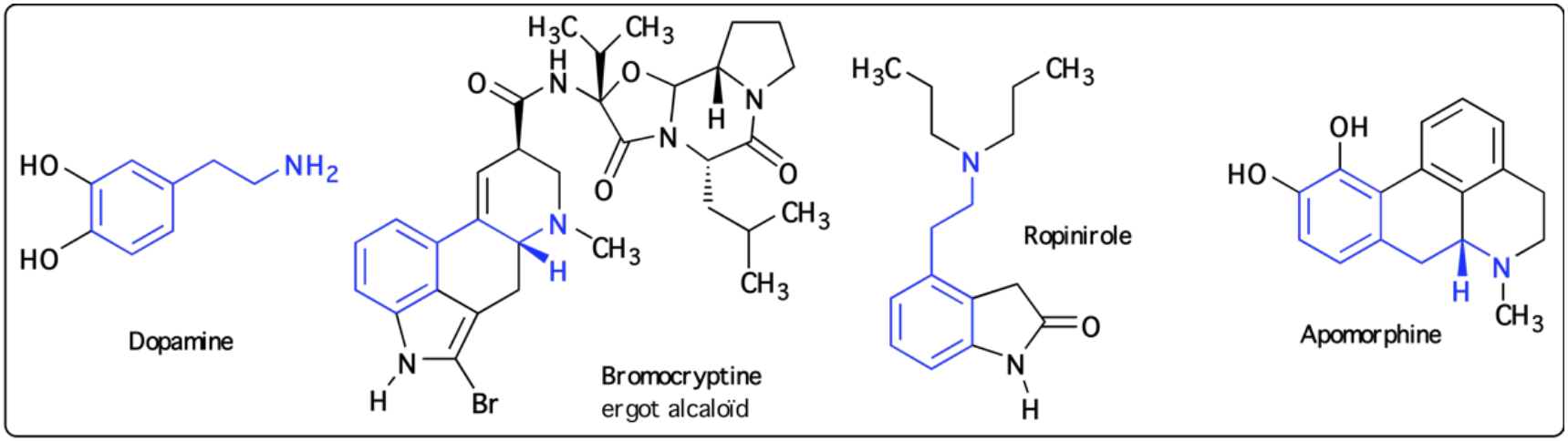
In a second time, comprehensive studies are performed in order to establish **structure/activity relationships**, and to realize **structural optimization** of identified hits.

Example 7 : rational development of cimetidine, and following « me-toos » :

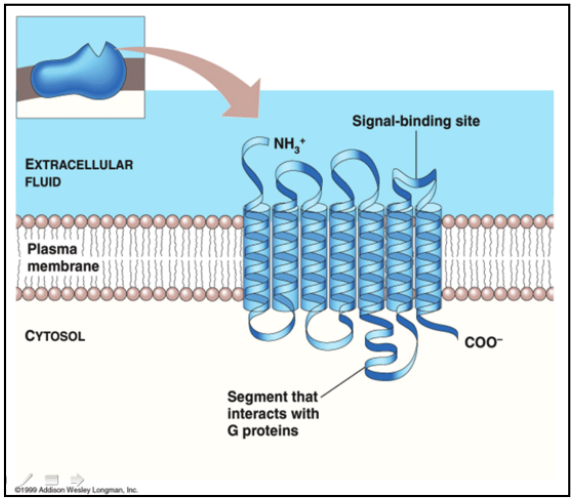


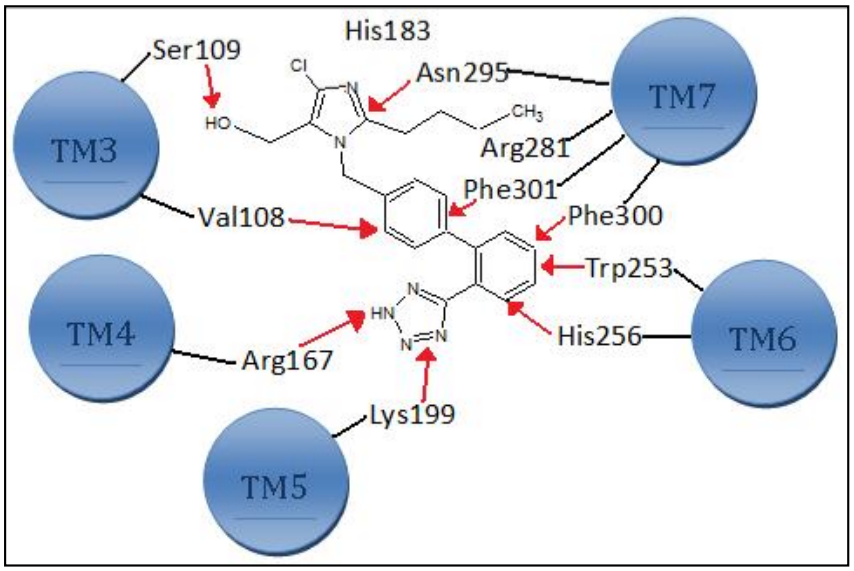


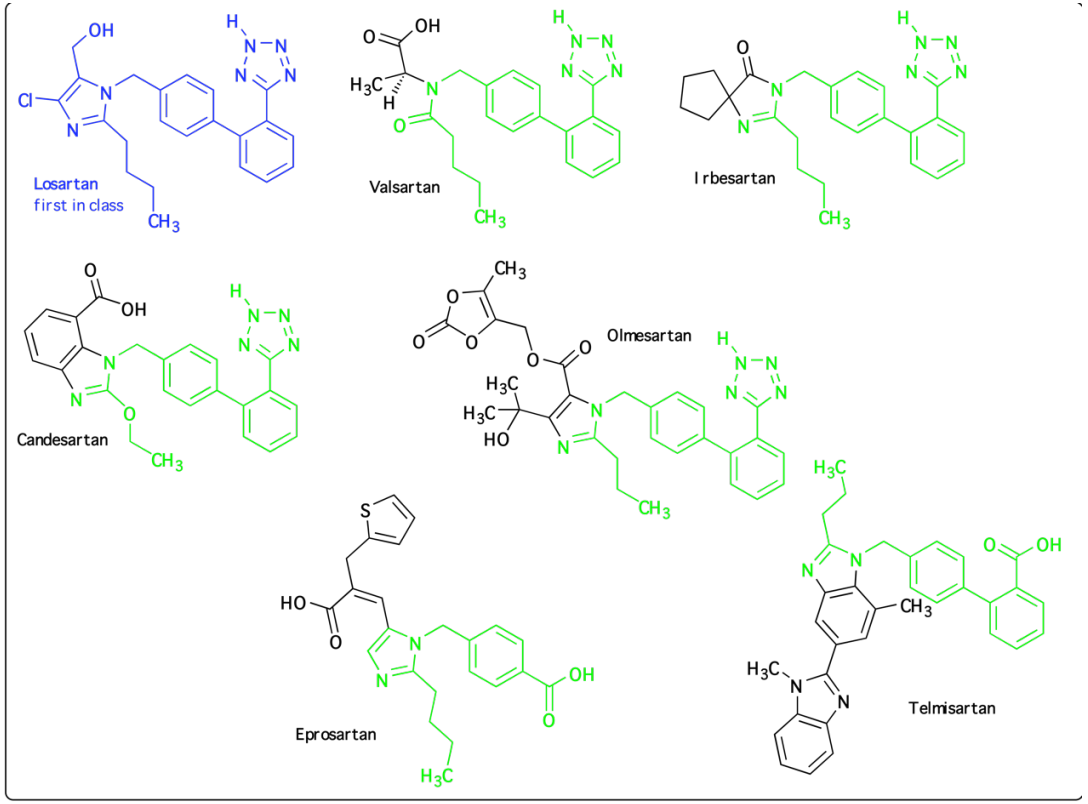
Example 7 : use of structure/activity relationships to identify the pharmacophoric moiety of D2 dopaminergic compounds :



Example 8 : *de novo*-conception of losartan, the first Angiotesin-II receptor antagonist, and following « me-toos » :







26 _ MULTITARGETED DRUG DISCOVERY

INTRODUCTION

Multitargeted drug = multitarget drug:

drug *acting on different targets*, implicated in same or different pathways.

Multitargeted drug therapy = polyparmacology:

use of *one drug* with *different mechanisms of action* to cure complex diseases.

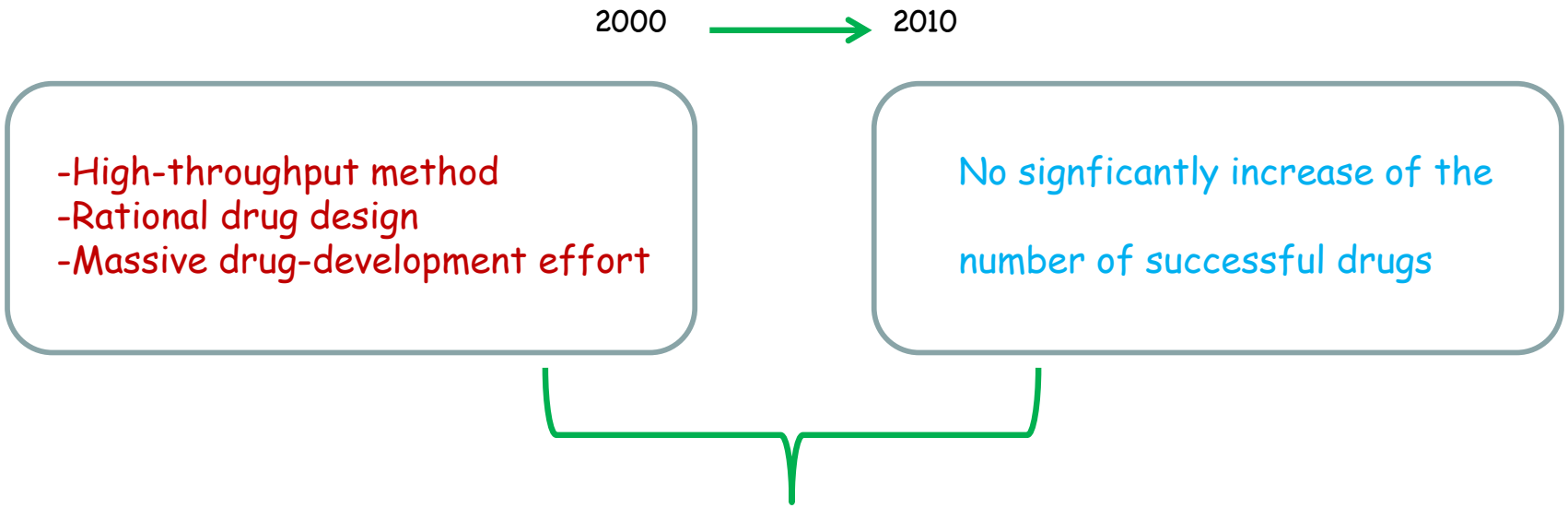
Crucial issues :

- *Affinity balance* between different target proteins and desired *in vivo* efficacy.

- Selection of *pharmacologically relevant targets* located on complementary pathological pathways.

- Identification of the *pharmacophoric* functions responsible for binding to targets.

The statement of fact:



Single-targeted drugs may not always induce the desired effect

Why?

A lot of reasons possible;
For example: development of compensatory ways (paralel or secondary signaling pathways, …)

Multitarget drugs *vs* combination therapy (global aspect of the problem)

Combination therapy = use of different drugs with different mechanisms of action to cure complex diseases

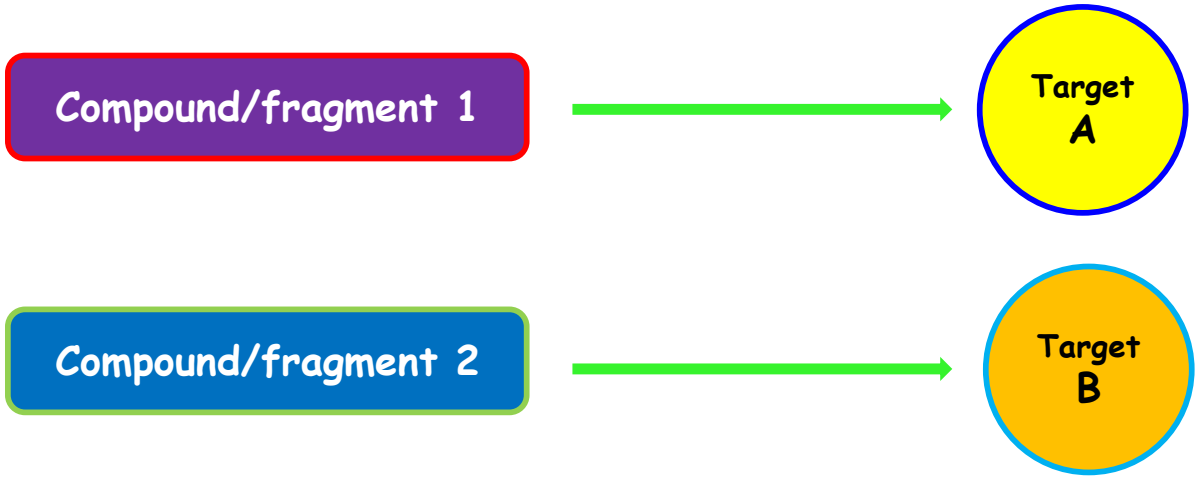
Multitarget drugs = use of one drug with different mechanisms of action to cure complex diseases.

Advantages of MT drugs =

- **additive effects** : target located on the same path;
- **synergic effects** : target located on functionally complementary pathways.

	Drugs combinations	Multitarget drugs
Drug-drug interactions	+	-
Regimen simplification	-	+
Patient compliance	-	+
PD and PK prediction and studies	complex	simpler
Drug doses		lower
Cost	higher	lower

The different multi-target drug design strategies : (ex.: dual-target design)



- **Chimera structure**
= conjugate structures

- **Fused structure**
= overlapping pharmacophores

- **Hybrid structure**
= integrated multitarget drug

In-silico methods for searching and design multi-target drugs

***In-silico* methods** have been widely explored for facilitating lead discovery against individual targets;

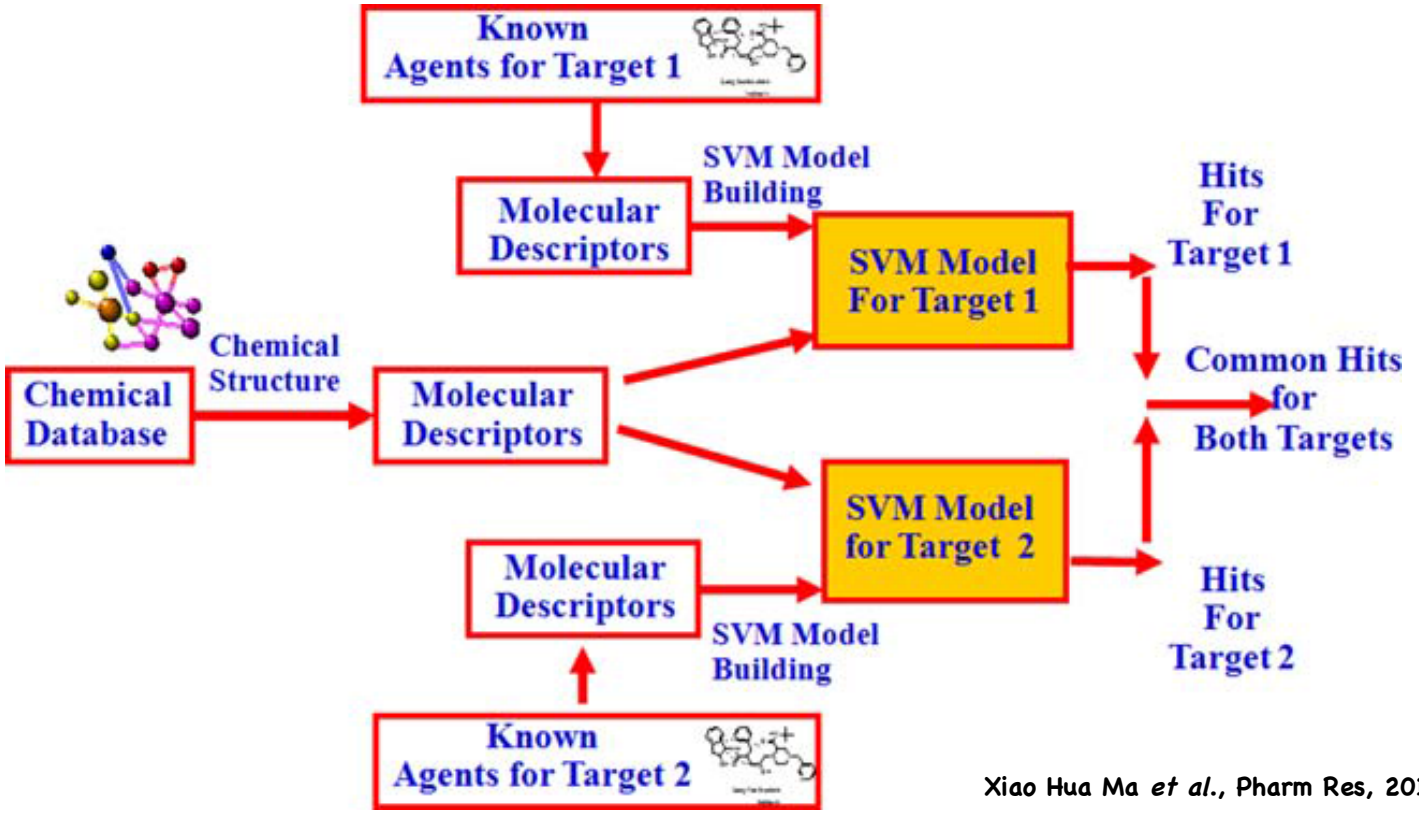
Particularly, we can quote :

- molecular and/or pharmacophore docking;
- structure-activity relationships (mt-SAR);
- quantitative structure-activity relationships (mt-QSAR);
- machine learning;
- combination methods.

These methods are classified into *combinatorial approaches* and *fragment-based approaches*.

Combinatorial approaches conduct parallel searches against each individual target to find virtual hits that simultaneously interact with multiple targets.

Illustration of molecular docking strategy for multi-target inhibitors, using virtual screening on Support Vector Machines (SVM) :



Xiao Hua Ma *et al.*, Pharm Res, 2010

Fragment-based approaches combine multiple elements of structural frameworks or multiple fragments (which have been introduced as tools for the design of multi-target agents) that bind to each individual target to design compounds that bind to multiple targets.

Illustration of framework combination approach

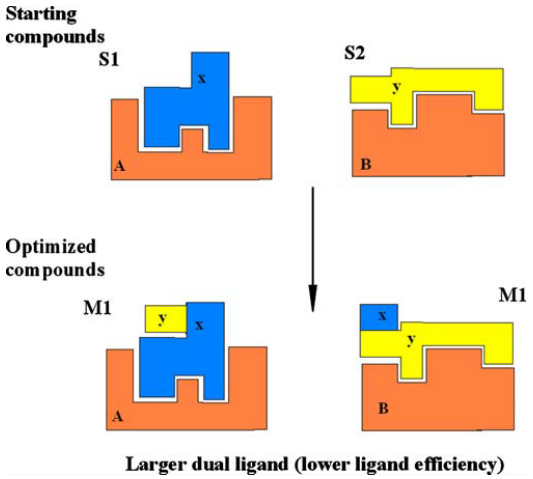
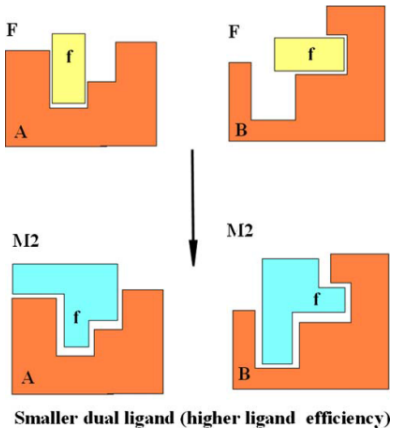


Illustration of fragment-based approach



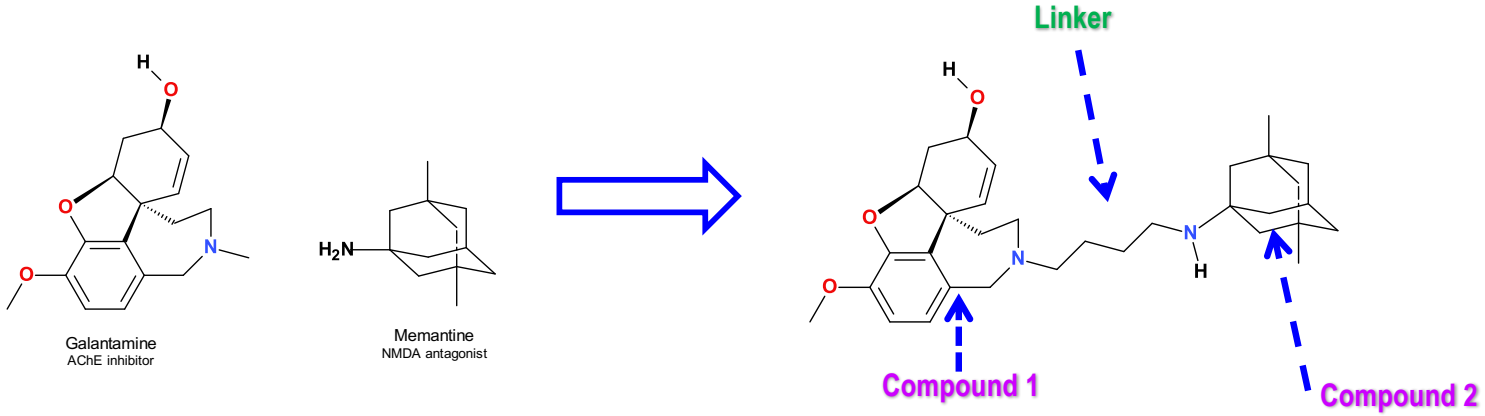
In one approach, the structure-activity relationships against individual targets are analyzed to find molecular fragments and essential binding features which are either combined or incorporated into active agents against selected multiple targets.

In another approach, molecular fragment libraries are searched to find the fragments with certain levels of activity against selected multiple targets, and the identified fragments are further optimized into more potent, bigger-sized multi-target active agents.

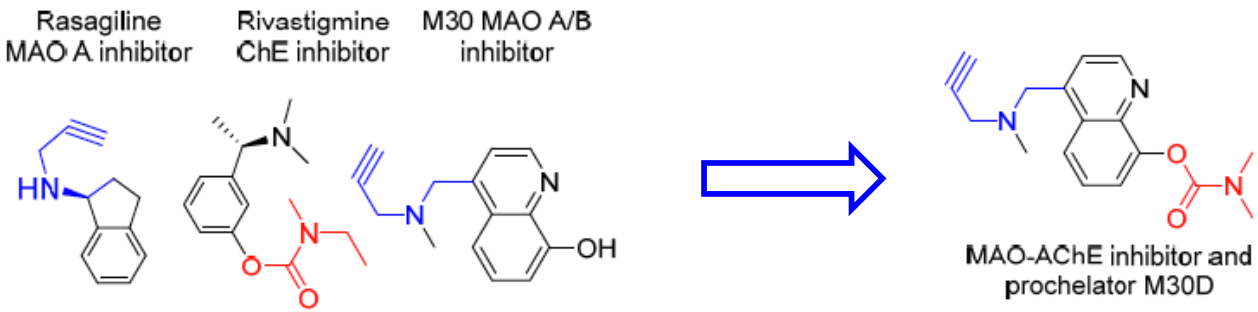
STUDY OF CASES

Anti-Alzheimer desease drugs (Zheng *et al.*, *Pharmaceuticals* , **2014**, *7*, 113-135) :

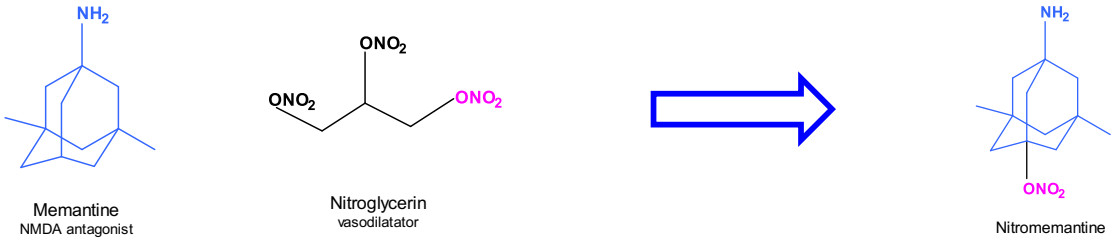
Chimera structure



Fused structure

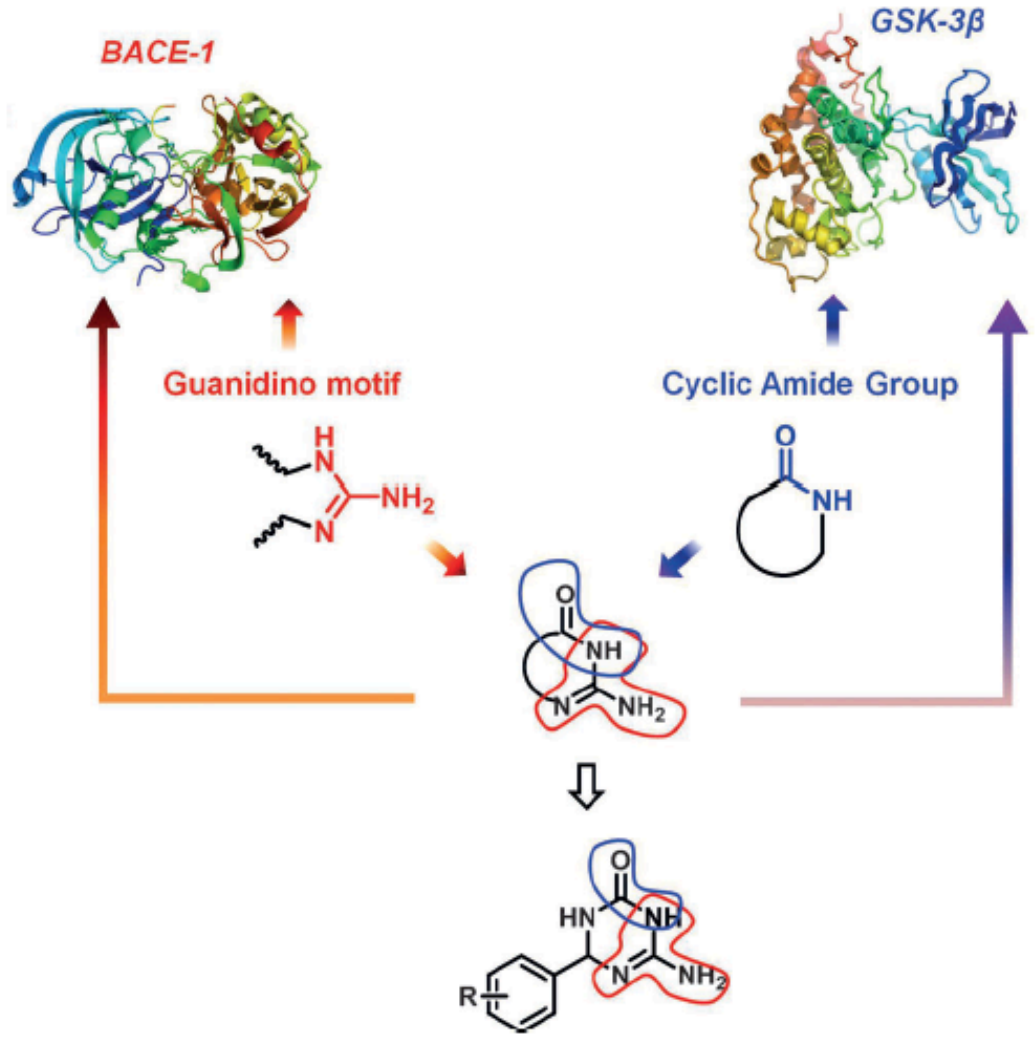


Hybrid structure



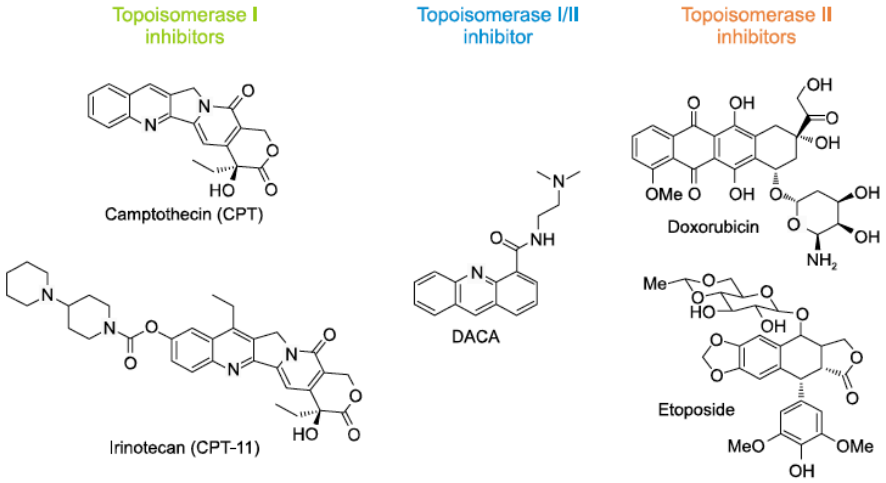
Design of anti-Alzheimer dual-inhibitors BACE-1/GSK3β using a fragment-approach strategy

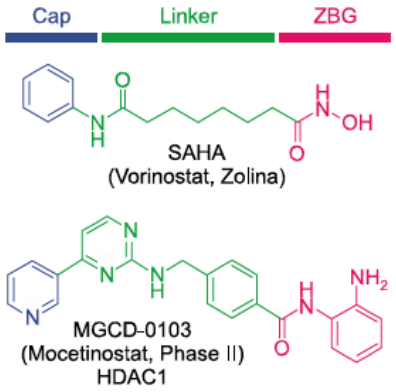
(Seo, J. Canc. Prev., **2015**, 20, 2, 85-91)

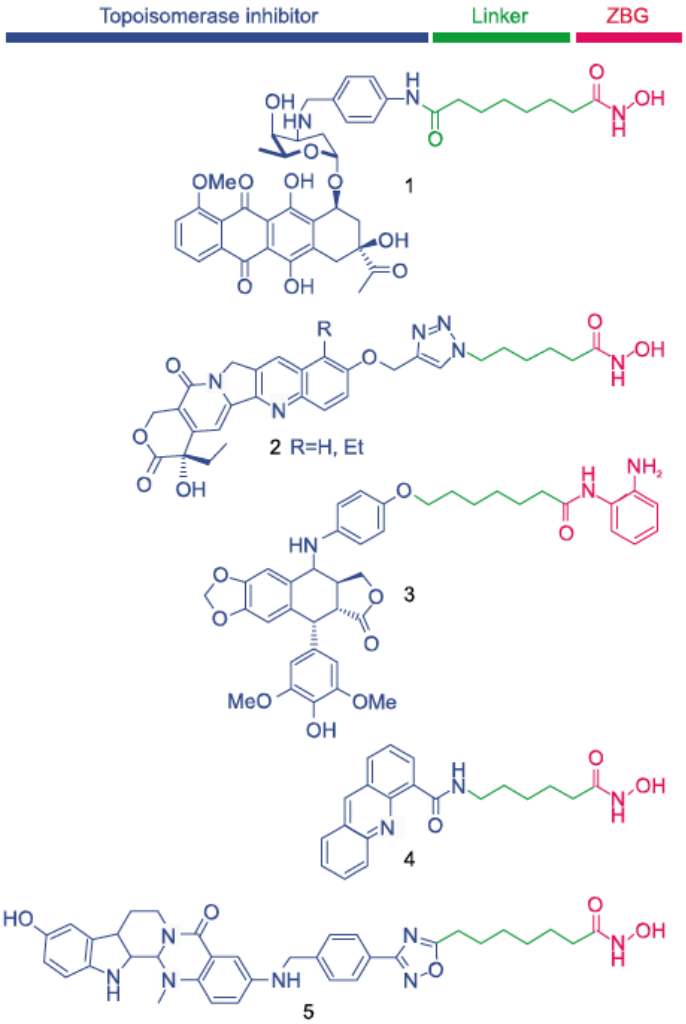


Anti-cancer drugs : dual inhibitors against topoisomerases and HDACs

(Prati *et al.*,Angew. Chem. Int. Ed., **2015**, 54, 1578 –1582)

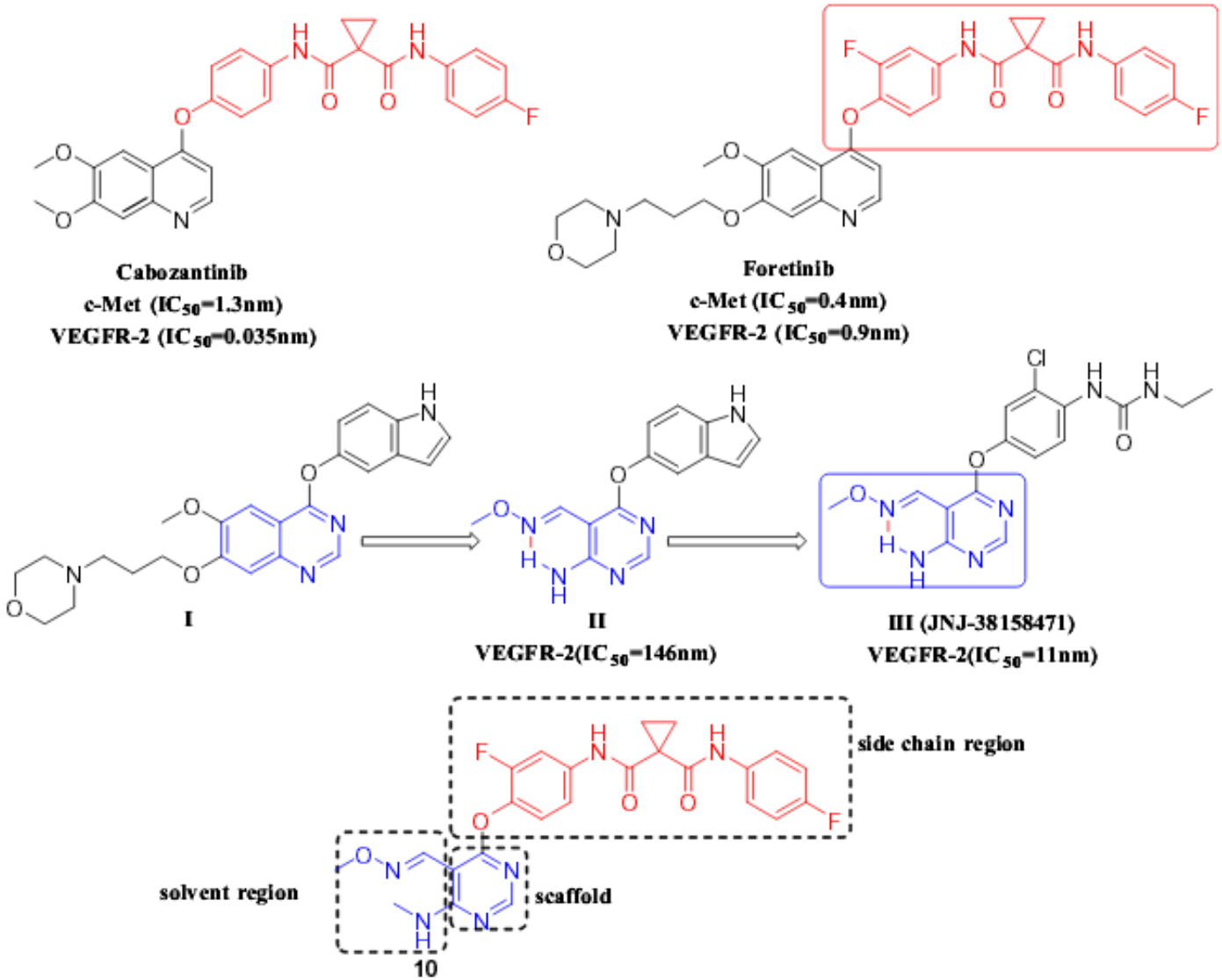






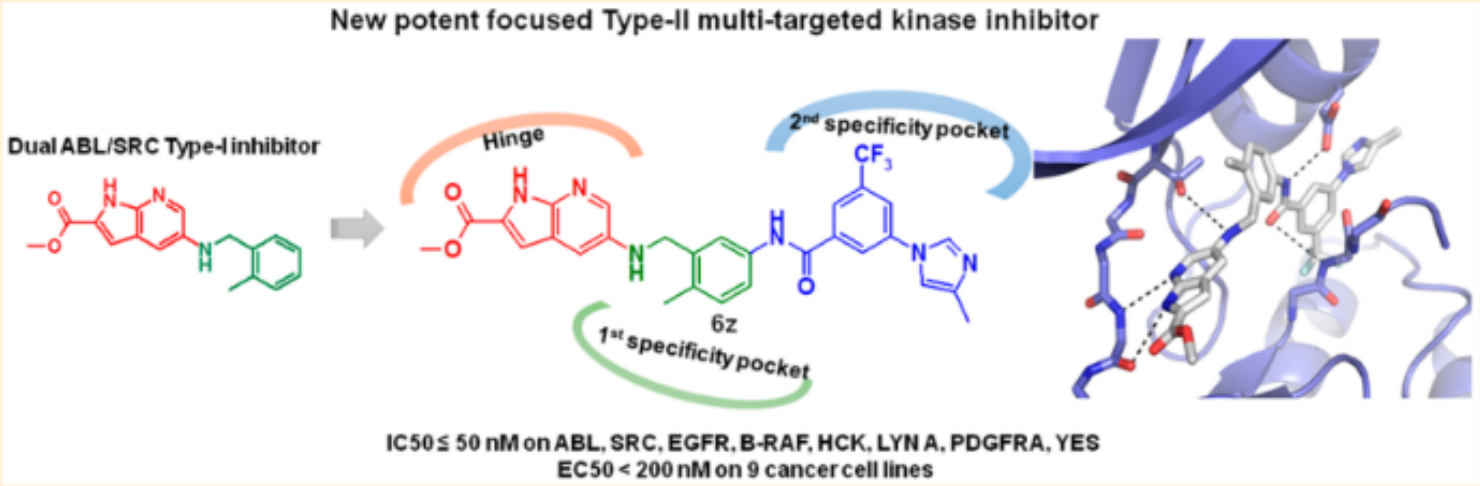
Anti-cancer drugs : Design of dual inhibitors of c-Met and VEGFR-2

Hao Qiang, Weijie Gu, DanDan Huang, Wei Shi, Qianqian Qiu, Yuxuan Dai, Wenlong Huang, Hai Qian, ***Bioorganic & Medicinal Chemistry***, **2016**, 24, 3353-3358.



Anti-cancer drugs : Rational design, synthesis, and biological evaluation of 7-azaindole derivatives as potent focused multi-targeted kinase inhibitors

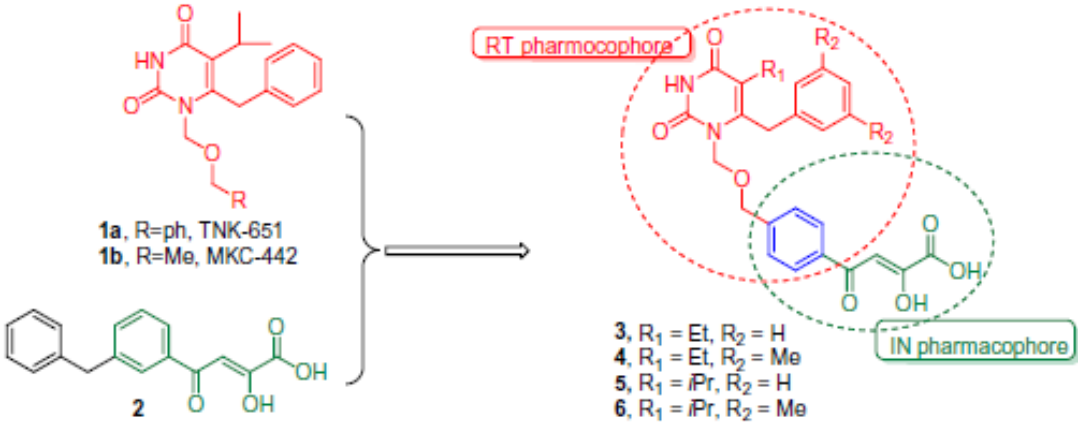
B. Dayde-Cazals et al., ***J. Med. Chem.***, **2016**, 59, 3886–3905.



ABSTRACT: Efforts were made to improve a series of potent dual ABL/SRC inhibitors based on a 7-azaindole core with the aim of developing compounds that demonstrate a wider activity on selected oncogenic kinases. Multi-targeted kinase inhibitors (MTKIs) were then derived, focusing on kinases involved in both angiogenesis and tumorigenesis processes. Antiproliferative activity studies using different cellular models led to the discovery of a lead candidate (**6z**) that combined both antiangiogenic and antitumoral effects. The activity of **6z** was assessed against a panel of kinases and cell lines including solid cancers and leukemia cell models to explore its potential therapeutic applications. With its potency and selectivity for oncogenic kinases, **6z** was revealed to be a focused MTKI that should have a bright future in fighting a wide range of cancers.

Anti HIV drugs : dual inhibitors of reverse transcriptase and integrase

Shuang-Xi Gu , Ping Xue , Xiu-Lian Ju , Yuan-Yuan Zhu, ***Bioorganic & Medicinal Chemistry***, **2016**, 24, 5007–5016.

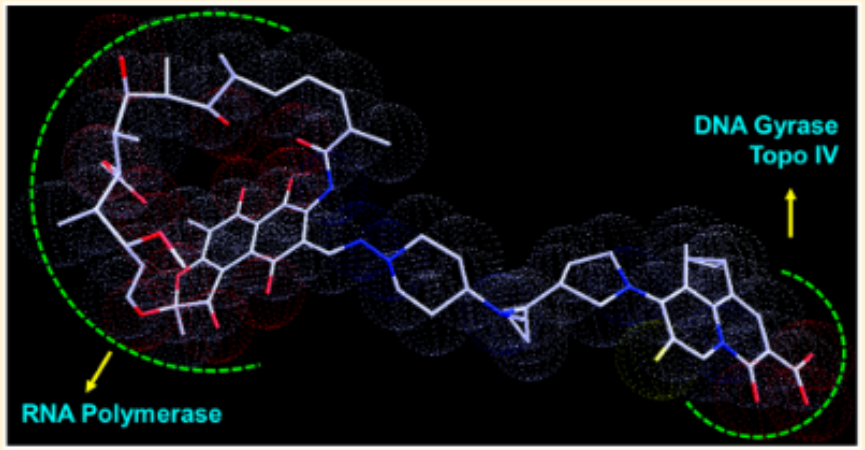


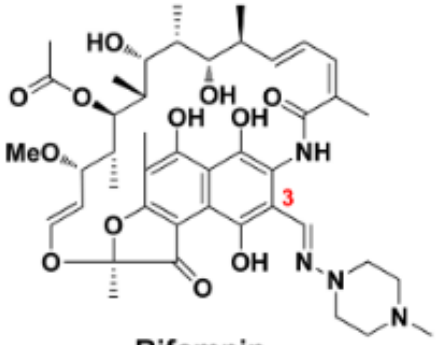
Inhibitor	RT IC_{50} (μM)	IN IC_{50} (μM)	HIV-1 EC_{50} (μM)	HIV-1 CC_{50} (μM)	TI (μM)
3	0.23	1.8	0.052	>10	>190
4	0.024	4.4	0.0097	>10	>1000
5	0.057	2.4	0.033	>10	>310
6	0.0092	7.7	0.017	>10	>600
1a	0.016	>100	0.016	>10	>610
2	>100	0.093	0.16	>10	>61

Anti bacterial drugs : Development of a Dual-Acting Antibacterial Agent (TNP-2092) for the Treatment of Persistent Bacterial Infections.

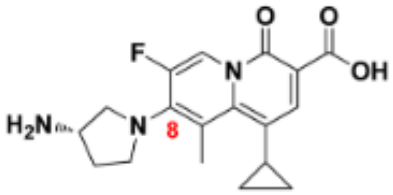
Z. Ma and A.S. Lynch, ***J.Med.Chem.***, **2016**, 59, 6645–6657.

ABSTRACT: The clinical management of prosthetic joint infections and other persistent bacterial infections represents a major unmet medical need. The rifamycins are one of the most potent antibiotic classes against persistent bacterial infections, but bacteria can develop resistance to rifamycins rapidly and the clinical utility of the rifamycin class is typically limited to antibiotic combinations to minimize the development of resistance. To develop a better therapy against persistent bacterial infections, a series of rifamycin based bifunctional molecules were designed, synthesized, and evaluated with the goal to identify a dual-acting drug that maintains the potent activity of rifamycins against persistent pathogens and at the same time minimize the development of rifamycin resistance. TNP-2092 was identified as a drug candidate and is currently in an early stage of clinical development for the treatment of prosthetic joint infections.

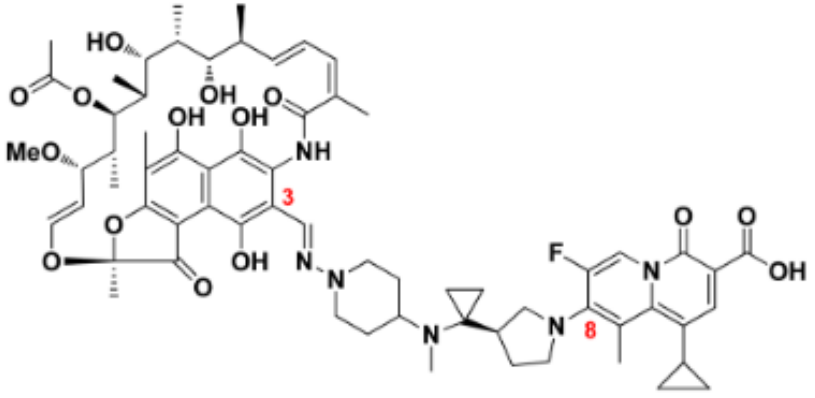




Rifampin



4 (ABT-719)



1 (TNP-2092)